PROCEEDINGS

OF THE

SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE

VOL. 32. APRIL, 1935. NO. 7.

Peiping Section

Peiping Union Medical College, January 30, 1935.

7934 C*

Positive Wassermann Reaction Induced in Rabbits by Injection of Hamster Tissues.

C. K. HU, DOROTHY H. WONG AND LOUISE PEARCE.

From the Division of Dermatology and Syphilology, Department of Medicine, and the Department of Bacteriology and Immunology, Peiping Union Medical College.

In connection with a previous study of the susceptibility of the Chinese hamster *(Cricetulus griseus)* to syphilitic infection,[1] it was noted that normal rabbits developed strongly positive Wassermann reactions after being injected with hamster tissues in which the syphilitic virus could not be demonstrated by rigid biological methods. The question arose as to whether the tissues of the normal hamster, when injected into the rabbit, are capable of inducing Wassermann reactive bodies in the circulating blood. Consequently, further studies were made on rabbits injected with various tissues of normal hamsters. The following is a summary of the results obtained with injections of various tissues by different routes.

Unless stated otherwise the hamster tissues used were emulsified in sterile normal saline shortly before being injected into the rabbit.

* C represents a complete, P a preliminary manuscript.

1 Hu, C. K., and Pearce, L., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1154.

Wassermann tests were made on the blood serum according to the Kolmer technique.[2] This method proved to be quite reliable in our hands. Among 71 normal rabbits tested there was only one false positive reaction in an animal which was in poor physical condition.

1. *Testis.* Seventeen rabbits received 0.5-1.0 cc. of the emulsion intratesticularly, and all of these developed a positive Wassermann reaction. In one rabbit injected intraperitoneally the same positive result was obtained. Two other animals injected subcutaneously into the right flank, gave similar results. Two rabbits injected intravenously with the emulsion which had been filtered through sterile gauze, in doses of 0.3 and 0.5 cc., showed a weakly positive reaction in the blood.

2. *Brain.* All 7 rabbits injected intratesticularly with the emulsion developed a strongly positive Wassermann reaction, while 2 other animals injected intravenously gave negative results.

3. *Whole blood* (not treated with saline). Each of 5 intratesticularly injected rabbits developed a fairly strongly positive Wassermann reaction. In another animal, injected subcutaneously (0.2 cc.), the reaction was weakly positive.

4. *Spleen.* Only one animal was injected subcutaneously (1.0 cc.). The reaction proved to be strongly positive.

5. *Kidney.* Two rabbits were injected (1.0 cc.) subcutaneously. The Wassermann reaction became positive in both cases.

Of the 5 varieties of hamster tissues used, the testis gave the strongest positive Wassermann reaction, and the blood gave the weakest response. The strongest reactions were obtained with the intratesticular route of injection, and the weakest with the intravenous route. Usually the Wassermann response was manifest from 2 to 3 weeks after the injections.

The substance (or substances) in the tissues of the normal hamster responsible for the induction of a positive Wassermann reaction in the injected rabbits has not yet been identified. Experiments carried out by Zia and Hu[3] showed that heterophile antigens present in the hamster tissues are not responsible for the positive Wassermann reaction.

The results of the experiments are conclusive in showing that the injection of normal hamster tissues induces a positive Wassermann reaction in rabbits. The response is independent of the route of injection.

---

[2] Kolmer, J., Infection, Immunity and Biologic Therapy, Philadelphia, W. B. Saunders, 1925, p. 478.

[3] Zia, S., and Hu, C. K., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 991.

7935 C

Heterophile Antigenicity of Hamster Tissues and Its Relation to Wassermann Reaction Induced in Normal Rabbits.

SAMUEL ZIA AND C. K. HU. (Introduced by C. E. Lim.)

From the Department of Bacteriology and Immunology and the Division of Dermatology and Syphilology, Department of Medicine, Peiping Union Medical College.

The finding of Hu and his coworkers[1] that normal rabbits receiving injections of hamster tissue developed strongly positive Wassermann reactions suggested the following 2 lines of study, namely, (1) the heterophile antigenicity of various hamster tissues, and (2) the relation of its antibody to the Wassermann reaction induced in rabbits. The presence of heterophile antigens in various tissues of experimental animals such as horses, guinea pigs, mice, etc.,[2, 3] as well as those of plants (corn)[4] has been demonstrated and the induction of the Wassermann reaction in rabbits by injections of extracts of guinea pig tissue and of lipoidal substances from rabbit testis has been observed.[5, 6, 7] In this study observations were made on the presence of heterophile antigens and Wassermann stimulating substances in the hamster tissues, and on the relationship between the two.

Methods. Ten rabbits were selected for the experiments. Tests were made on the blood serum of these animals for the presence of "natural" heterophile antibody. Wassermann tests were also performed on the same blood specimens. The results of these tests were all negative. Various hamster tissues, emulsified in sterile normal saline, were injected into 8 rabbits, either intratesticularly or subcutaneously; 2 remaining rabbits received intratesticular injections of an emulsion of guinea pig testis. Following these injections, a determination of heterophile antibody and a Wassermann test were made simultaneously at weekly intervals on the blood of these animals for a total period of 5 weeks.

---

1 Hu, C. K., Wong, Dorothy Huie, and Pearce, Louise, PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 989.

2 Bull, C. G., Newer Knowledge of Bacteriology and Immunology, University of Chicago Press, Chicago, 1928, p. 933.

3 Iijima, T., *Scien. Rep. Gov. Inst. Inf. Dis.*, 1921, **1**, 97.

4 Hyde, R. R., Chapman, J., and Kiesling, C., *Am. J. Hyg.*, 1934, **20**, 465.

5 Taniguchi, T., *Scien. Rep. Gov. Inst. Inf. Dis.*, 1921, **1**, 87.

6 Eagles, H., *J. Exp. Med.*, 1932, **55**, 677.

7 Klauder, J. V., *Arch. Derm. Syph.*, 1931, **23**, 884.

For the determination of heterophile antibody, the usual technique was followed. Various dilutions (from 1:80 to 1:1280) of inactivated sera in 1 cc. amounts were mixed with 0.5 cc. of guinea pig complement (2 units) and 0.5 cc. of 2% sheep cells, making a total volume of 2 cc. Controls without complement were set up with the highest concentration of serum (1:80) at the same time. Readings were taken at the end of 2 hours' incubation in a water bath at 37°C. For the Wassermann test, the Kolmer method[8] was employed. The results are presented in Chart 1.

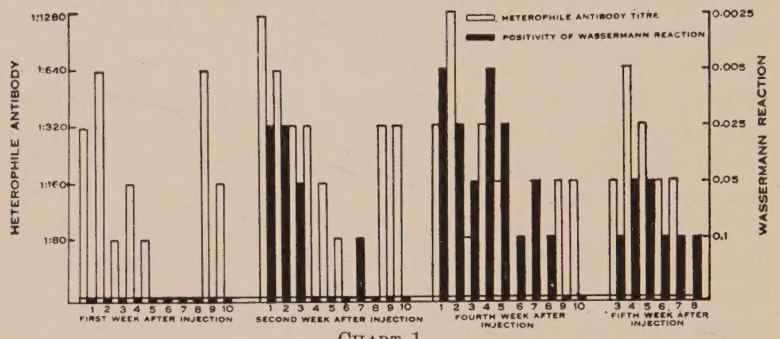


CHART 1.

The Wassermann reaction and heterophile antibody response in rabbits to intratesticular and subcutaneous injections of various hamster and guinea pig tissues.

Rabbit 1 and 2 received one cc. of an emulsion of hamster testis injected intratesticularly.

Rabbit 3 and 4 received one cc. of an emulsion of hamster testis injected subcutaneously.

Rabbit 5 and 6 received one cc. of an emulsion of hamster kidney injected subcutaneously.

Rabbit 7 received one cc. of an emulsion of hamster spleen subcutaneously.

Rabbit 8 received two-tenths cc. of hamster whole blood injected subcutaneously.

Rabbit 9 and 10 received one cc. of an emulsion of guinea pig testis injected intratesticularly.

The height of the columns represents the highest dilution of serum in which the tests were positive.

In order to go further in establishing the absence of any relation between these 2 bodies, absorption tests were carried out as follows: Ground hamster, guinea pig and rabbit tissues were separately mixed with 2 rabbit sera showing high heterophile antibody titres and giving strongly positive Wassermann reactions. The mixtures were placed in a water bath at 37°C. for 2 hours and then centrifugalized. The supernatant sera were retested. The results are presented in Table I.

Results. From Chart 1, it is clear that the injection of emulsions of hamster testis, either intratesticularly or subcutaneously, and of

[8] Kolmer, J. A., Infection, Immunity and Biologic Therapy, W. B. Saunders, Philadelphia, 1925, p. 478.

TABLE I.

Titre of heterophile antibody and degree of Wassermann reaction in rabbit serum before and after absorption with various tissues.

	Heterophile antibody determination serum dilution						Wassermann reaction quantity of serum					
	1:80	1:160	1:320	1:640	1:1280	Control 1:80	.1	.05	.025	.005	.0025	Control .1
Rabbit I												
Unabsorbed serum	++	++++	++++	++++	+++	0	++++	++++	++	0	0	0
Absorbed with rabbit kidney	+++	++++	+++	+++	+++	0	+++	++	0	0	0	0
Absorbed with hamster testis	±	±				0	++++	++++	0	0	0	0
Rabbit II												
Unabsorbed serum	+++	+++	+++	++	+	0	++++	++++	+++++	+++	0	0
Absorbed with rabbit kidney	+++	+++	++	+	+	0	++++	++++	+	0	0	0
Absorbed with guinea pig kidney	0	0				0	++++	++++	+	0	0	0
Absorbed with hamster testis	0	0				0	++++	++++	+++++	0	0	0

hamster kidney or spleen subcutaneously, induced in the appearance of both heterophile antibodies and Wassermann reactive bodies in the rabbit's blood. That these 2 bodies are not identical is indicated by the fact that one week after injection the heterophile antibodies were present in large quantity, while the Wassermann reactive bodies were absent. The heterophile antigenic power seemed to be greater with testicular tissue than with that of the kidney or spleen, and the intratesticular route of injection was somewhat more effective than the subcutaneous one. The same differences obtained in the production of Wassermann reactive bodies. It is to be noted, however, that the intratesticular injection of guinea pig testicular emulsion into 2 rabbits produced a fairly high titre of heterophile antibody, but that the Wassermann test was negative with 0.1 cc. of these sera during the observation period of 5 weeks.

Table I shows that the heterophile antibodies may be completely absorbed by either hamster testicle or guinea pig kidney. After absorption, the quantity of Wassermann reactive bodies was not materially reduced in comparison with that of the sera absorbed with rabbit's tissue which contains no heterophile antigen.

In another group of 8 rabbits which received intratesticular injections of syphilitic rabbit testis, the Wassermann reactions became strongly positive, but heterophile antibody was not detectable.

Summary and Conclusion. The presence of heterophile antigen in hamster tissues was demonstrated by direct examination for the antibodies in rabbits injected with various hamster tissues. This was confirmed by the absorption test with guinea pig kidney. That there is no relationship between heterophile antibody and a positive Wassermann reaction, induced in normal rabbits by injections of the hamster tissues, was demonstrated by the following facts. In the first place, contrary to Taniguchi's statement,[9] it was found that although guinea pig tissue has a high heterophile antigenic power, it does not induce a positive Wassermann reaction in the normal rabbit. Secondly, after complete absorption of heterophile antibody from rabbit serum, the positivity of the Wassermann reaction is not materially reduced. Thirdly, tests made on 8 syphilitic rabbits, infected intratesticularly by injecting syphilitic rabbit testicular emulsion, showed that the sera developed strongly positive Wassermann reactions, but were negative for heterophile antibody.

[9] Taniguchi, T., *J. Path. Bact.*, 1921, **24**, 222.

7936 C

Local Skin Reactivity in Rabbits to an Extract of Ascaris Lumbricoides.

JUI-WU MU. (Introduced by C. N. Frazier.)

From the Division of Dermatology and Syphilology, Department of Medicine, Peiping Union Medical College.

Since Shwartzman[1] reported that a local skin reaction characterized by pronounced hemorrhagic necrosis could be induced by intravenous administration of the culture filtrate of *B. typhosus* 24 hours after intracutaneous injection of the same filtrate, many workers have confirmed his observation. It has been noted,[2] however, that this local skin reactivity cannot be obtained by intravenous injections of various non-bacterial substances following intradermal preparation with the same material. The present communication deals with the reproduction of this phenomenon with the extract of *Ascaris lumbricoides.*

The extract of *Ascaris lumbricoides* was prepared by putting 4 gm. of the whole ascaris worm in a mixture of one part of Coca's solution[3] and 3 parts of normal saline for 2 weeks, during which time the mixture was shaken from time to time. After the extraction, the mixture was filtered through one layer of ordinary filter paper, and 0.25 cc. of concentrated phenol was added to 100 cc. of the filtrate. Before the extraction, the ascaris worm was washed in running water, immersed in 10% formalin solution for 10 minutes, and then again washed in running water, following which it was dried at room temperature and ground into a fine powder.

Altogether 45 adult male albino rabbits were used, 20 of them being employed as test animals and 25 as controls. Twenty-four hours prior to each experiment, the skin over the flank was shaved and the hair was brushed with only ordinary soap. The test rabbits were injected intradermally with 0.1 cc. of ascaris extract and 24 hours later treated intravenously with 3 cc. of the same extract per kilogram of body weight. The control rabbits were divided into 3 groups. The first group, 10 animals, were injected intradermally with 0.1 cc. of ascaris extract and 24 hours later treated intravenously with 3 cc. of a mixture of one part of Coca's solution and 3 parts of normal saline with 0.25% of phenol per kilogram of

1 Shwartzman, G., Proc. Soc. Exp. Biol. and Med., 1928, **25**, 560.

2 Shwartzman, G., *J. Exp. Med.*, 1930, **51**, 571.

3 Coca, A. F., and Milford, E. L., *J. Immunol.*, 1925, **10**, 555.

body weight. In the second group, 10 animals were injected with 0.1 cc. of ascaris extract and 24 hours later nothing was given intravenously. In the third group, 5 animals were injected intradermally with 0.1 cc. of a mixture of one part of Coca's solution and 3 parts of normal saline with 0.25% of phenol, and 24 hours later nothing was administered intravenously. All the intradermal injections were given with a sharp tuberculin needle and a tuberculin syringe calibrated to 0.01 cc., and were made equally deep in the epidermis. In the case of test animals and the first group of the control animals, the sites of intradermal injections were examined 24 hours after the intradermal preparation and 4 hours after the intravenous injections. In the case of the second and third groups of the control animals, the sites of intradermal injections were examined 24 hours and 28 hours after the intradermal injections had been given. The skin of the sites of intradermal injections was removed from the test and control animals 28 hours after the preparatory intradermal inoculations for histological study.

Results. Twenty-four hours after the preliminary intradermal injections of ascaris extract, erythematous papules appeared in all test animals and those of the first and second groups of the control animals. The size of this primary reaction varied from 2 to 5 cm. in the test animals, 4 hours after the intravenous injections, the erythematous skin became extremely dark blue and swollen in the center with a deep red zone at the periphery in 8 of them and moderately so in 7 of them. The remaining 5 animals showed no detectable reaction. The discolored skin resembled closely a severe bruise. The intensity and size of the discoloration was not related to the intensity and size of the erythema produced by the preparatory intracutaneous inoculations. Microscopically, the sections of the skin exhibiting frank hemorrhage showed very marked exudation, massive infiltration of polymorphonuclear neutrophil leucocytes, pronounced *endovascular* necrosis, partial obliteration of blood vessels by thrombosis and hyalinization, extensive hemorrhage in the subcutaneous tissues and rupture of blood vessels. Sections of the skin of the negatively reacting rabbits revealed mild edema with slight migration of polymorphonuclear neutrophil leucocytes, and moderate dilatation and congestion of blood vessels. No signs of endovascular necrosis, rupture of blood vessels or hemorrhage were found. Regarding the control animals, no gross alteration was observed in the erythematous papules 4 hours after the intravenous injections in the first group. In the second group, the erythematous papules remained unchanged in appearance, when they were exam-

ined 28 hours after the preparatory intradermal injections. In the third group, no reaction whatever was noticed both 24 hours and 28 hours after the intradermal injections. Microscopically, the sections of the skin removed from the first and second groups of control animals showed about the same changes as those of the negatively reacting test animals. In the sections of the skin of the third group of control animals, there were no abnormal findings except for a mild edema in the subcutaneous tissues.

Summary. Intradermal injection of rabbits with an extract of *Ascaris lumbricoides*, followed 24 hours later by intravenous administration of the same extract, produced hemorrhagic necrosis which grossly and microscopically conformed with that described by Shwartzman.

7937 P

Development of Female Characteristics in Adult Male Rabbits Following Prolonged Administration of Estrogenic Substance.

C. N. FRAZIER AND J. W. MU.

From the Division of Dermatology and Syphilology, Department of Medicine, Peiping Union Medical College.

Estrogenic substance was prepared by extracting acidulated human pregnancy urine with butyl alcohol in a continuous extractor as described by Veler, Thayer, and Doisy.[1] The alcohol was removed by distillation, using a vacuum pump, and the residue dissolved in ethyl ether. The ether extract was added to olive oil, and the ether evaporated. The resulting olive oil solution was then assayed for its estrogenic content by the technique of Coward and Burn.[2] The quantity required to produce estrus in 50% of 20 ovariectomized, sexually mature, albino rats constituted the rat unit used in the following experiment.

Twenty-four male albino rabbits of known parentage, 16-17 months of age, were employed. Eight of these were injected subcutaneously once a day, 6 days a week, with from 20-60 rat units of estrogenic substance in olive oil. Injections were continued in 6 animals for 250 days or more. Eight rabbits were injected in like

1 Veler, C. D., Thayer, S., and Doisy, E. A., *J. Biol. Chem.*, 1930, **87**, 357.

2 Coward, K. H., and Burn, J. H., *J. Physiol.*, 1927, **63**, 270.

manner with plain olive oil, and 8 were left untreated. All animals had a syphilitic infection given in connection with another experiment in which they were being used. Only those rabbits receiving injections of estrogenic substance showed the variations from the normal described below.

Mammary glands. Hypertrophy of nipples to the size of those of the lactating female rabbit developed after 80 days of treatment. Milk could be expressed from the nipples. In 6 animals, the secretion increased in amount and became thick and white, in 2 animals it remained thin and watery. After continuing for at least 90 days in all animals, lactation decreased, and the nipples became smaller. Subsequently, milk could be expressed only from a few nipples in most of the animals, and in one case it disappeared entirely after being present for 120 days. Four rabbits continued to secrete some milk for as long as 200 days. Lactation did not depend upon the stimulation of secretion by withdrawal of milk. Enlarged mammary glands and dilated superficial blood vessels were visible under the shaved skin. The abdominal skin looked thinner and smoother than normal.

The lactating males willingly fostered young rabbits, and in 2

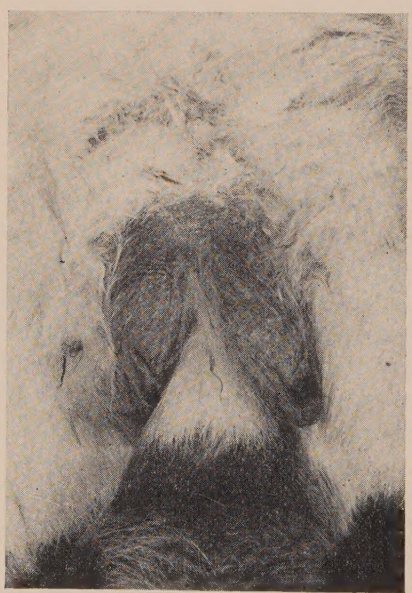


FIG. 1.
The scrotal sacs of an untreated rabbit.

FIG. 2.
The scrotal sacs (covered with hair) of a rabbit treated with estrogenic substance.

instances suckled them. Three animals 8 days old survived under these conditions for 5-6 days, which was 1-2 days longer than 3 controls.

External genitalia. The testes became atrophic after 100 days of treatment. Decrease in size was subsequently progressive; in 6 animals treated for 250 days the testes were less than half their original size, and were held high in the scrotum at the inguinal ring. In this position some were fixed. With the atrophy and ascent of the testis, the scrotum shortened. The usual smooth surface of its dependent portion disappeared, leaving a shallow sac covered with hair.

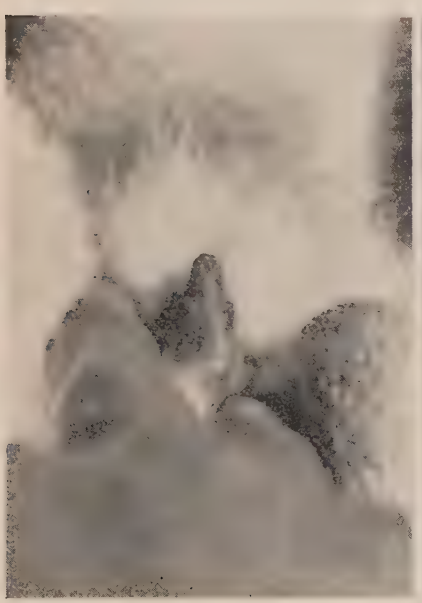


FIG. 3.
The penis of an untreated rabbit.

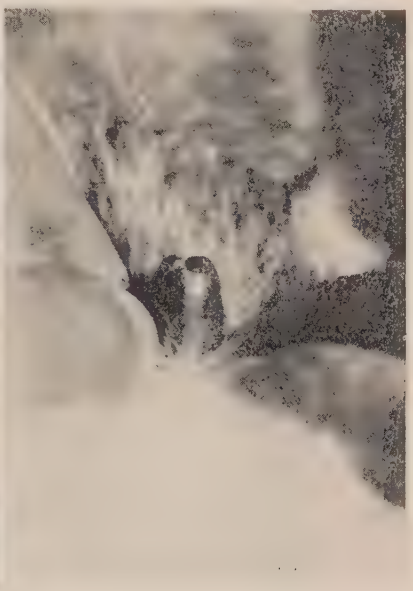


FIG. 4.
The penis of a rabbit treated with estrogenic substance. The prepuce is everted showing a light longitudinal band, which is the atrophied cavernous bodies.

After 144 days of treatment, the glans penis softened and the prepuce became swollen. The urinary meatus elongated as dorsal cleavage and shortening of the glans penis rapidly progressed. Within a period of 3 weeks, the urethra had receded into the depths of the prepuce and the glans penis had entirely disappeared, leaving the atrophied cavernous portion of the penis which tapered distally to a narrow band on the inner ventral surface of the prepuce. At

this stage the prepuce, penis and urethra of 7 animals resembled the female pudenda with its clitoris and common urogenital vestibulum.

Skin and Hair. The coats of the feminized males were unusually heavy and clean except over the areas where the injections were given. After 200-235 days of treatment the skin under the chin became loose and redundant, encircling the anterior neck like a ruff. A similar formation of the skin may be found in normal female rabbits, especially after they have borne young.

FIG. 5.
The neck of an untreated male rabbit.

Behavior. The feminized males were more passive and docile than the normal male rabbits except while fostering young ones, when they frequently resisted by biting. On repeated trials they were indifferent toward females, and evaded normal males who were persistent in attempting copulation. In one case copulatory actions

FIG. 6.
The fold of skin on the anterior cervical region of a male rabbit treated with estrogenic substance.

were terminated in ejaculation of semen by the normal male, but no spermatozoa could be found in the vestibulum-like structure of the receptive animal.

7938 P

Rate of Formation of Acetylcholine in Placenta In Vitro.

HSI-CHUN CHANG.

From the Department of Physiology, Peiping Union Medical College.

We have demonstrated that placental acetylcholine originates in the syncitial layer of the villus, and that it exists in a free form.[1]

[1] Wen, I. C., Chang, H. C., and Wong, A., to be published.

We now have evidence that it also exists in a reserve form. The former can be readily extracted by alcohol, and quite satisfactorily by saline, providing precaution is taken to prevent the activity of cholinesterase with sufficient eserine and handling at ice-cold temperature. The latter appears to be liberated on incubation of the placenta at a favorable temperature, *viz.*, 37°C. The present report deals with the rate of liberation of this reserve acetylcholine from its precursor *in vitro*.

Five experiments have given similar results, one of which will be briefly presented. In this particular experiment, a placenta which was freed from its foetal membrane and cord was minced and divided into 18 lots each weighing 20 gm. Three series of observations were carried out, one with eserine represented by EC, E11, E12, E13, E14, and E15, and 2 without designated by C, 11, 12, 13, 14, 15 and C′, 11′, 12′, 13′, 14′, 15′; each series thus consisted of 6 samples. Three cc. eserine solution (25 mg. eserine salicylate) were added to each sample of the eserine series, while 3 cc. saline were added to each of the non-eserine series before starting the experiment. The control samples of the 3 series were immediately extracted for their free acetylcholine with 2 volumes of 96% alcohol in the ice chest for 17.5 hours. The rest were incubated at 37°C., one sample from each series being removed at hourly intervals for similar extraction of the same duration. At the end of extraction, the tissues were squeezed through gauze, the weight of residue being taken and the filtrate refiltered and measured before storage on ice. The rectus test[2] was used for assay. Fig. 1 shows the complete record of the assay of these 18 samples on the same eserinized rectus against the same acetylcholine chloride standard, while Fig. 2 shows the result plotted against time.

FIG. 1.

[2] Chang, H. C., and Gaddum, J. H., *J. Physiol.*, 1933, **79**, 255.

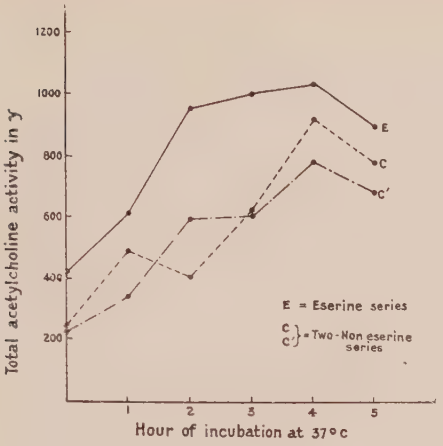


FIG. 2.

The liberation of reserve acetylcholine increases during incubation and reaches a maximum at the 4th hour, the average hourly increment during the first 4 hours being 169, 138 γ acetylcholine activity for the 2 non-eserine series respectively. Under the protection of eserine, the initial free acetylcholine activity was higher but the average increment during incubation was only 119 γ per hour.

Elucidation of the mechanism and significance of the present finding in relation to our previous claim[3, 4] constitutes some of the objectives of the experiments which are now in progress.

7939 P

In Vitro Experiments on the Viability and Excystment of Paragonimus Cyst.

O. K. KHAW. (Introduced by R. J. C. Hoeppli.)

From the Division of Parasitology, Department of Pathology, Peiping Union Medical College.

Cysts were obtained from infected crabs (*Potamon denticulatus* Milne-Edwards) collected from Lan Ting, where the first 2 human cases of paragonimiasis in China were reported by Ying.[1]

Experiments were carried out in hollow glass slides in which

[3] Chang, H. C., and Wong, A., *Chinese J. Physiol.*, 1933, **7**, 151.

[4] Wong, A., and Chang, H. C., *Chinese Med. J.*, 1933, **47**, 987.

[1] Ying, Y. Y., *Nat. Med. J. China*, 1930, **16**, 638.

actively motile encysted cercariae were placed in the medium to be tested. Freed cysts which had been kept in ice chest for 7 days could live in wine made from millet (*Sorghum vulgare* L.) in (a) 10% alcohol, for 43 hours at 22°C., and 20 hours at 37°C., (b) 25% alcohol, for 1 hour at 22°C., and (c) 50% alcohol for a few seconds at the same temperature. In yellow rice wine with 14% alcoholic content, death took place in 18 hours at 22°C., and in 15 hours at 37°C.* They could be kept alive in 10% formalin for 23 days and in 0.9% saline for a like period in the ice chest (10°C.).

The metacercariae caused to excyst by the methods described in the next series of experiments were actively motile in trypsin 1% plus sodium carbonate 0.2% for at least 3 hours at 37°C. if bacterial growth was not checked and could remain dormant for 43 hours in the ice chest in the same medium. When they were placed alternately at 22°C. and at 16°C. they could live 84 hours. In 12% bile and in artificial intestinal juice plus bile (5%), their viability was 42 hours in the ice chest.

These experiments demonstrate that in a diluted millet wine containing 10% alcohol and in rice wine (14% alcohol), the encysted metacercariae were viable up to 43 and 18 hours at room temperature (22°C.), respectively, and that they could be kept alive in the ice chest (10°C.) in 10% commercial formalin or in 0.9% saline for over 3 weeks. Therefore, the customary mode of preparing crabs, as practiced by the villagers in the endemic area where the infection rate for crabs varies from 25 to 100%, by soaking them, very often only over night at room temperature so as not to spoil the taste, in a weak solution of salt and yellow rice wine seasoned with spices, cannot kill all the cysts of Paragonimus. This would account for the high rate of infection, 87% in one village (Chen and Rose),[3] prevailing in the Lan Ting district.

The early Japanese investigators believed that, under natural conditions, the excystment took place spontaneously. However, Yoshida[4] showed that this was not the case. Kobayashi[5] thought that gastric or intestinal juice alone did not cause hatching, as the larvae were seen escaping from the cysts when transferred from the crabs

* If these experiments show discrepancies with those of Ameel[2] with alcohol, they may be due to the fact that the wines contain ethereal and other extracts which may modify its action.

[2] Ameel, D. J., *Am. J. Hyg.*, 1934, **19**, 299.

[3] Chen, W. L., and Rose, G., Ninth Congr. Far East. Assn. Trop. Med. Abst., 1934, 62.

[4] Yoshida, S., *J. Parasit.*, 1916, **2**, 175.

[5] Kobayashi, H., *Mitteil. Med. Hochsch. Keijo*, 1921, **4**, 5.

to tap water, normal saline, or a higher temperature. Ameel[6] did not obtain consistent results by the successive uses of artificial gastric and pancreatic juice at 37°C. In order to test this point, the following experiments were made *in vitro*.

Paragonimus cysts measuring from 0.336 to 0.506 mm. with a wall thickness of 12 to 20 microns, containing actively motile metacercariae were suspended at 37°C. in the following media with the results noted. (1) In artificial gastric juice there was no excystment after 5½ hours and larvae were dead in 15 hours; (2) in 0.2% HCl, they died in 18 hours and the cyst walls in some were broken; (3) in dog's or artificial gastric juice for 3 hours, the larvae remained quiescent but became restlessly active on being transferred to an artificial intestinal juice made up of trypsin 1% plus 0.2% sodium carbonate, and excysted in 3¾ through a triradiate opening in the intact wall; (4) in artificial intestinal juice plus 5% fresh cow's bile *without* previous contact with gastric juice, the excystment took place in 1¾ hours; (5) in the same medium but *without* the bile, the larvae escaped in *between* 45 to 90 minutes; (6) in bile alone, there was excystment in 75 minutes in 12% dilution, and in 2¾ hours in 1.5%, 6%, and 100%; (7) In Na_2CO_3 (0.2%) alone, the larvae remained dormant for 24 hours and died after 3 hours' immersion in artificial intestinal juice; (8) no change was observed in pure or diluted *boiled* bile, the larvae being still motile at the end of 13 hours; or in *boiled* artificial intestinal juice in which the larvae were found alive after 6 hours but dead in 16 hours.

To determine the rôle played by digestive active of the alimentary tract, experiments with dead cysts were made with the result that there was no visible solution or dissolution of the cyst wall when cysts were immersed in dog's gastric juice or artificial intestinal juice for 58 hours. Some cysts burst after 18 hours in 0.2% HCl; and in 0.4% Na_2CO_3, they turned black and appeared hard and brittle and the walls of some of them gave way. Observations on empty cyst walls kept in artificial intestinal juice, renewed every third day, showed that some of them did not totally disappear even after 4 weeks.

The process of excystment does not differ from that of *Clonorchis sinensis* (Faust and Khaw[7]) and *Opisthorchis felineus* (Vogel[8]) except that the larva makes its escape long before the true outer cyst wall has been demonstrably and visibly digested off.

---

[6] Ameel, D. J., *Am. J. Hyg.*, 1934, **19**, 279.

[7] Faust, E. C., and Khaw, O. K., Studies on *Clonorchis sinensis*, *Am. J. Hyg.*, 1927, Monogr. Series B.

[8] Vogel, H., *Zoologica*, 1934, **33**, Heft 86.

7940 C

Pour Plate Study of Bacteriophage.

ALBERT C. H. YEN. (Introduced by C. E. Lim.)

From the Department of Bacteriology and Immunology, Peiping Union Medical College, Peiping.

A simple method for direct and accurate enumeration of bacteriophage is still lacking. In many instances it is desirable to determine the bacteriophage unit directly. For this purpose 2 general methods are now in use, (1) the counting of plaques formed when bacteriophage and susceptible bacteria are spread on the surface of agar plates, and (2) the determination of the highest dilution in broth for a given bacteriophage to cause complete lysis of susceptible bacteria. The best agreement by the dilution method of titration has been calculated by Clark[1] to be 60%. The difficulty of spreading evenly on an agar surface and the adsorption of a variable amount of bacteriophage by the spreader are the disadvantages of the streak method. If bacteriophage and susceptible bacteria are mixed thoroughly in meat infusion agar and then poured into plates, we find that the technique is not only simplified but its accuracy is also increased. It seems worthwhile, therefore, to test the practicability of the pour plate method and the optimal conditions for the demonstration of bacteriophage plaques under various factors of growth.

A dysentery Shiga bacteriophage isolated from a single plaque was used. Pour plates were made by mixing 1 cc. of diluted bacteriophage, 0.5 cc. of an 18-hour agar slant growth of susceptible bacteria in concentration of 1:50 in saline and 15 cc. of 2% meat infusion agar (pH 7.6). Upon incubation at 37°C. for 24 hours, 2 kinds of plaques appear: (a) surface plaque, which is large, clear, and extending through the depth of the medium, and, (b) deep plaque, which is small, less clearly outlined, and situated below the surface.

In agreement with Bronfenbrenner and Korb[2] we found the size of the individual plaque to be bigger as the concentration of agar is decreased (Fig. 1). It was also found that the number of visible plaques differed within 10% when the agar concentration used varies from 0.5 to 2%. When, however, the concentration of agar

[1] Clark, H., *J. Gen. Physiol.*, 1928, **11**, 71.

[2] Bronfenbrenner, J. J., and Korb, C., *J. Exp. Med.*, 1925, **42**, 483.

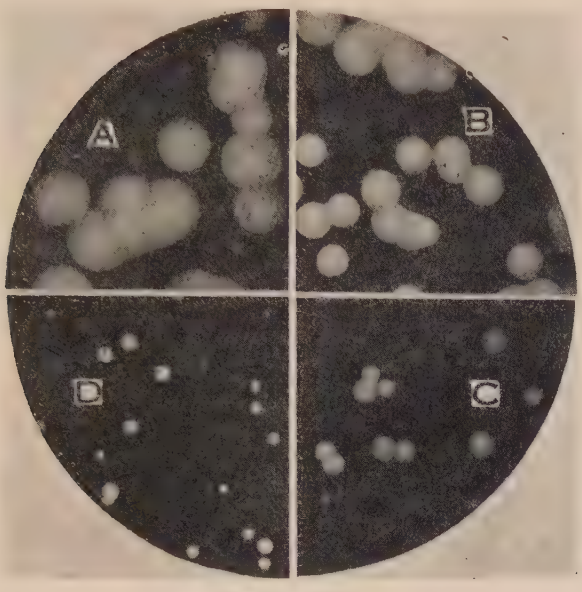


FIG. 1.

Showing size of plaques in different concentrations of agar using 1 cc. of a 1×10^{-7} dilution of bacteriophage (A) 0.5% agar, (B) 1% agar, (C) 2% agar, (D) 3% agar, natural size.

is increased over 2%, the number of plaques became considerably lower and finally no plaques could be detected when 5% agar is employed. The plaques are found to be distinct and large when the concentration of the susceptible bacterial suspension is between 1×10^9 and 5×10^9 organisms per cc. When the susceptible bacteria is decreased to 5.0×10^7 organisms per cc., the plaque appears markedly less clear cut and smaller in size and in number.

The thickness of media in the plate is also found to influence markedly the size of the plaque. In general, the thinner the plate the bigger is the plaque. When 5 to 10 cc. of medium were poured to a glass plate of 15 cm. diameter, practically all plaques are large, clear and extending to the surface, but when 15 to 30 cc. of medium were poured the average size of the plaque diminishes, and many plaques are situated below the surface so that counting becomes difficult.

When plates are incubated at 20°C. for 24 hours or grown anaerobically the plaques are smaller and less distinct (Fig. 2, A and D) than those incubated at 37°C. for 24 hours. It appears from these experiments that the slower the rate of bacterial growth, the smaller is the size of the plaque. In making the dilution of bac-

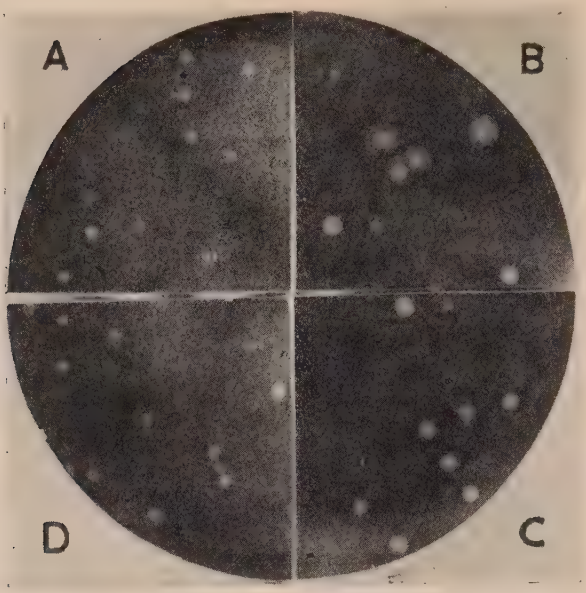


FIG. 2.
Showing plaques and ring formation (A) in 2% agar, 20°C., aerobic, 24 hours, (B) in 2% agar at 37°C. for 48 hours, concentric rings of lysis around plaques, (C) similar plate as B, but the media is thicker than in B, no ring around plaques, (D) in 2% agar at 37°C. anaerobic for 24 hours, natural size.

teriophage for plaque counting, it was found that meat infusion broth (pH 7.6) serves as a better diluent than saline which tends to give a lower count.

Since these results show that size and number of bacteriophage plaques in pour plates may vary greatly depending upon percentage of agar, concentration of susceptible bacteria, nature of diluent, and thickness of plates, the pour plate method can not be used for accurate quantitative determination of bacteriophage unless optimal conditions are observed. Such conditions are obtained when the following procedure is adopted: 1 cc. of bacteriophage diluted in meat infusion broth (pH 7.6) and 0.5 cc. of an 18 hour agar slant growth of susceptible bacteria in concentration 1:50 in saline are placed in a glass plate of 15 cm. diameter and 10 cc. of 1.5% meat infusion agar is added; the contents in the plate are thoroughly mixed and incubated at 37°C. aerobically for 24 hours before the plaques are counted.

When the plaque counts were determined in a serial dilution from undiluted bacteriophage, it was found that the number of plaques produced are not directly proportional to the respective concentra-

tion of bacteriophage used. The results are in agreement with Dreyer,[3] who also found that a relatively higher figure is obtained when higher dilution is used. Our results are given in Table I, which illustrates the applicability of Dreyer's standard curve showing the relation of bacteriophage units and plaque count. The K calculated shows a close agreement.

TABLE I.

Bacteriophage dilution, factor, B	1/B x 10^5	Plaques counted	Units from Dreyer's curve	K	B x plaques
1 x 10^6	.1	750	1337	7.310 x 10^{-5}	750 x 10^6
2	.05	450	652	7.669 "	900 "
4	.025	265	315	7.937 "	1060 "
8	.0125	155	154.5	8.090 "	1240 "
16	.00625	86	74.14	8.430 "	1376 "
32	.003125	51	39.30	7.952 "	1632 "
64	.001563	27	19.13	8.171 "	1728 "
128	.0007813	14	9.56	8.173 "	1792 "
256	.0003906	7	4.79	8.155 "	1792 "

Bacteriophage plaques in the poured plates presented another interesting phenomenon (Fig. 2, B and C). In some of the plaques it was found to be surrounded by 2 to 4 concentric rings. The first ring is a narrow zone of partial clearing immediately next to the perfectly clear central plaque. The second ring is a zone of comparatively dense bacterial growth. The third ring is another zone of partial clearing more opaque than the first ring and a little wider. The fourth ring is a zone of dense bacterial growth thicker than the surrounding parts of homogeneous bacterial growth in the plate. Often not all of these rings are conspicuous, but two rings, namely, a central plaque surrounded by a ring of dense bacterial growth which is in turn encircled by a very narrow zone of partial clearing may be seen. In this case the first ring can not be distinguished from the central plaque and the fourth ring is absent. The width of these rings varies according to the percentage of agar employed. It is wider when the percentage of agar is low. The occurrence of these rings depends upon several factors. It is seen when inoculum of susceptible bacteria is heavy (5×10^9 organisms per cc.) and the media is poured thin (5 to 8 cc. of agar in a plate of 15 cm. diameter). It is better seen when incubated aerobically at 37°C. for 2 to 4 days. They are absent when incubated at 20°C. or anaerobically.

The mechanism in the production of these rings is not clear.

[3] Dreyer, C., and Campbell-Tenton, M. L., *J. Path.*, 1933, **36**, 399.

Bronfenbrenner[4] stated that the diffusion of bacteriophage occurred even at 4°C. This diffusion factor in itself seems to us inadequate to account for the occurrence of rings of lysis. The following theory is presented as a possible explanation. The central plaque ceases to increase in size after 24 hours because by then, some inhibitory product resulting from bacterial lysis might have been accumulated. If one may be permitted to assume that this inhibitory product cannot diffuse in the agar as rapidly as the bacteriophage, then one might expect a concentric ring of partially lysed bacteria immediately surrounding the central clear plaque, to be surrounded by another zone of clearing. Thus alternate zones of clear and cloudy rings corresponding to presence and absence of bacteriolysis can be seen. The increased density of bacterial growth occurring at the outermost ring might be due to the stimulating action of the bacteriophage.

7941 P

Resistance In Vitro of Leishmania Donovani to Contamination with Bacteria.

LILY S. ZIA AND C. T. TENG. (Introduced by C. E. Forkner.)

From the Department of Medicine, Peiping Union Medical College.

Leishmania donovani, the causative agent of Kala-azar, is generally believed to be a delicate organism which cannot survive in the presence of common bacteria. The studies of Spagnolio[1] and those of Giugni and Benoni[2] tended to indicate that contamination with various common bacteria is detrimental to the culture of this parasite. The recent demonstration by Forkner and Zia[3, 4] of viable and infective *Leishmania donovani* in the midst of numerous bacteria in the oral and nasal secretions of 13 out of 14 patients suffering from Kala-azar has, however, led us to alter our concept con-

[4] Bronfenbrenner, J., "The Newer Knowledge of Bacteriology and Immunology," The University of Chicago Press, Chicago, 1928, p. 526.

[1] Spagnolio, G., *Malaria e Malat. d. Paesi Caldi,* 1912, **3**, 151. *Abs. Trop. Dis. Bull.,* 1912, **1**, 8.

[2] Giugni, F., and Benoni, F., *Malaria e Malat. d. Paesi Caldi,* 1915, **6**, 89. *Abs. Trop. Dis. Bull.,* 1915, **6**, 220.

[3] Forkner, C. E., and Zia, L. S., *J. Exp. Med.,* 1934, **59**, 491.

[4] Forkner, C. E., and Zia, L. S., *J. Exp. Med.,* 1935, in press.

cerning the resistance of this parasite to bacterial contamination and prompted us to test more specifically the exact relationship which exists *in vitro* between *Leishmania donovani* and some of the common bacteria with which they may be associated in the mammalian host.

The well-known and commonly employed N.N.N. (Novy-Mac Neal-Nicolle) medium is obviously unsuitable for our experiment, since the parasite will not grow or multiply in this medium in the presence of bacterial contamination. We found that a simple medium, with which Carter[5] successfully cultured *Leishmania tropica* from contaminated oriental sores, was suitable for our purpose. The medium used by us was prepared with slight modification and simplification, as will be reported in our final publication.

The *Leishmania donovani* used in the experiments were obtained from 2 sources. The flagellates were obtained from a 2 or 3 weeks old culture on N.N.N. medium. Leishman-Donovan bodies were obtained from the spleens of infected Chinese hamsters. The animals were killed with ether anesthesia and the spleens removed with aseptic technique. Emulsions were made by macerating approximately one-third or one-fourth of each of the spleens in 3 or 4 cc. of sterile 0.9% sodium chloride solution, and they were then ready for use.

The bacteria employed in the experiments were from stock and recently isolated cultures either in meat infusion broth or on China blue plate. They were *Staphylococcus aureus, Streptococcus hemolyticus, Pneumococcus* type undetermined, *Pneumococcus* type 1, and *B. coli.* In the case of *Staphylococcus aureus, Streptococcus hemolyticus* and *Pneumococcus,* 2 small platinum loopfuls of the broth cultures, and in the case of *B. coli,* 2 colonies from the China blue plate, were inoculated at about the same time as the *Leishmania donovani* into the respective tubes to get the desired contaminations. Both the inoculated and the control tubes were left in an incubator with the temperature maintained between 20° and 22°C. Examination of each tube for presence of flagellates was accomplished by removing a drop of the medium with a platinum loop. The drop was deposited on a slide, covered by a cover slip and examined under the microscope with a high dry lens.

We found that the Leishman-Donovan bodies may flagellate in Carter's medium after being associated, for at least as long as 41 hours, with the various types of bacteria except *B. coli,* and that the

[5] Carter, M., *Brit. Med. J.*, 1909, **2**, 647.

resulting flagellates can multiply and live for from 10 to 24 days in the original tubes without subculture. The contaminating bacteria were recovered from the respective tubes at the time when the flagellate growth was most abundant. These bacteria represent fairly well the kinds of bacteria with which *Leishmania* may come into contact when discharged from the body of their host. Our results, therefore, lend additional support to the hypothesis that the infectious agent of Kala-azar may be transmitted, at least in some cases, through the agency of the secretions from the upper respiratory and upper alimentary tracts.[3, 4] *B. coli* is the only bacterium in our group which exhibits a definite detrimental effect on the development and survival of the Leishman-Donovan bodies. Forty-one hours after inoculation, when the other tubes were showing Leishman-Donovan bodies with beginning flagellation and when in some there were already a few actively motile flagellates, there were only degenerating Leishman-Donovan bodies in the tubes contaminated with *B. coli*. It is suggested that under ordinary circumstances, the Leishman-Donovan bodies discharged from the body through the lower alimentary tract are quickly destroyed by the ever-present *B. coli*. But if the duration of the association of these 2 in the lower intestinal tract is shortened by increased peristaltic movements as in dysentery or diarrhea, or if the parasites are protected by flakes of mucus, it would seem reasonable to think that they may survive and may also serve as a source of infection.

Cleveland Section

Western Reserve University, March 8, 1935.

7942 C

A Method for the Estimation of Serum Iron.*

FRANKLIN C. BING AND RAMON F. HANZAL.

From the Department of Biochemistry, School of Medicine, and the Institute of Pathology, Western Reserve University.

The drawing of blood under conditions aiming to avoid hemolysis, as described by Fowweather,[1] has yielded in our hands serum that invariably gives a positive benzidine test and shows the absorption bands of oxyhemoglobin if a sufficient thickness of solution is examined. The present method for the estimation of the non-hemoglobin iron of blood serum consists of 2 steps, the analysis of the serum for total iron and for hemoglobin iron.

The total iron is determined by ashing 2 cc. of serum with 2 cc. of concentrated sulfuric acid, with the aid of 30% H_2O_2. The ashed sample is diluted to 15 cc. with water, enough potassium permanganate is added to give a permanent pink color, 5 cc. of ethyl acetate are layered over the solution and, finally, 5 cc. of 20% ammonium thiocyanate solution are added and the mixture is shaken. The color in the ethyl acetate layer is compared in a micro-colorimeter with a standard containing 0.005 mg. of iron similarly treated. The traces of iron in the reagents are determined by blank analyses.

The quantitative benzidine method[2] is employed for the determination of the hemoglobin, but allowance must be made for the effect of serum proteins on the reaction. Proteins and certain salts cause a diminution in the color produced in the benzidine acetate-

* The expenses of this investigation were defrayed in part by a grant from the Council on Pharmacy and Chemistry of the American Medical Association.

[1] Fowweather, F. S., *Biochem. J.*, 1934, **28**. 1160.

[2] Bing, F. C., *J. Biol. Chem.*, 1932, **95**, 387.

hemoglobin-H_2O_2 system. The method may be summarized briefly. To 2 cc. of the benzidine reagent 0.5 cc. of blood serum and 0.5 cc. of water are added, followed by 1 cc. of 0.6% H_2O_2 solution. In another test tube the same procedure is carried out, except that 0.5 cc. of the standard solution of blood, containing 0.05 mg. of hemoglobin per cc., is used in place of 0.5 cc. of water. The colors are allowed to develop and compared with a standard in the usual way.[2] If 40% of the hemoglobin added is recovered (the recovery varies from about 30 to 50% depending on the age of the reagents) the figure for the apparent hemoglobin content of the serum is multiplied by 2.5 to obtain the true value. Finally, the mg. of hemoglobin per 100 cc. of serum are computed as micrograms of iron by multiplying by the factor 3.35.

The validity of the hemoglobin method has been checked by spectroscopic examination of serum. The thickness of solution at which the α band of oxyhemoglobin just disappeared showed that the hemoglobin actually present was approximately 2 or 3 times more than the apparent concentration shown by the benzidine reaction. In addition, serum was treated with 30% H_2O_2 at room temperature until all the hemoglobin had been destroyed. The recovery of added hemoglobin was the same as the recovery of hemoglobin added to untreated serum.

TABLE I.
Iron Content of Blood Serum.

Specimen No.	Total Fe	Hb Fe	Non-Hb Fe	Inorganic Fe
1	330	44	286	270
2	333	33	300	330
3	375	47	328	320
4	365	17	348	350

All figures are reported in terms of micrograms of Fe per 100 cc. of serum.

Table I shows the results of analyses of 4 different samples of dog serum, drawn several hours after the oral administration of iron salts. Only 3 cc. of serum are required for a single determination of both total iron and hemoglobin iron. For comparison, estimations of the inorganic serum iron were made by the method of Tompsett,[3] which determines the ferrous iron in the protein-free filtrate of serum that first is treated with a reducing agent. This method requires 10 cc. of serum. The satisfactory agreement of the 2 sets of results indicates that the non-hemoglobin iron of blood serum is inorganic iron.

[3] Tompsett, S. L., *Biochem. J.*, 1934, **28**, 1536.

7943 P

Acid-Base Balance of Blood in Hyperthermia.

WAYNE H. DANIELSON AND ROBERT M. STECHER. (Introduced by V. C. Myers.)

From the Departments of Biochemistry and Medicine (City Hospital Division), School of Medicine, Western Reserve University, Cleveland.

It was shown some time ago that voluntary hyperventilation induces an elevation of the blood pH and a lowering of the bicarbonate concentration.[1, 2] Since fever, whether due to disease or produced by various physical means, is also accompanied by similar changes in the blood pH and bicarbonate concentration, hyperventilation has been suggested as the cause of the disturbance and the condition is considered to be one of a primary CO_2 deficit.[3] From the observations of Peters, Bulger, Eisenman and Lee[4] one infers that in instances of hyperventilation, the extent of the electrolyte changes depend upon the duration and intensity rather than the cause and nature of the hyperpnea.

Hyperthermia produced by means of the Kettering Hypertherm has been employed by one of us (R.M.S.) in the treatment of certain diseases. It is common for the patients receiving this form of therapy to perspire profusely and to consume considerable quantities of fluid. Some individuals prefer to drink water while others prefer a dilute salt solution. Occasionally some of the patients show somewhat violent systemic disturbances. The present investigation was undertaken to observe the differences in the total acid-base balance of the blood and salt concentration of the sweat when water or a dilute salt solution was drunk by the patient.

This is a preliminary report of the total acid-base balance changes of the serum obtained in 4 experiments on a single individual. In 2 of the experiments the patient was allowed to drink water and in the other 2 a 0.6% solution of sodium chloride. The body temperature was controlled at an average of 40°C. The serum acid-base changes observed when water was given were an elevation of the pH, a fall in the CO_2 tension and a fall in the bicarbonate, chloride, and total

1 Grant, S. B., and Goldman, A., *Am. J. Physiol.*, 1920, **52**, 209.

2 Davies, H. W., Haldane, J. B. S., and Kennaway, E. L., *J. Physiol.*, 1920, **54**, 32.

3 Peters, J. P., and Van Slyke, D. D., Quantitative Clinical Chemistry: Interpretations. Williams and Wilkins Co., 1932, pp. 954, 990.

4 Peters, J. P., Bulger, H. A., Eisenman, A. J., and Lee, C., *J. Biol. Chem.*, 1926, **67**, 175.

base concentrations. The total determined acid decreased more than the total base so that there was an increase in the undetermined acid. These same changes were observed whether the hyperthermia was carried out for 2 or 4 hours. A recovery specimen taken 2 hours after the short term experiment showed a fall in pH below the control level, a further fall in the chloride and total base concentrations, a partial recovery of the CO_2 tension and bicarbonate concentration, and a complete return of the undetermined acid to its control level.

Similar changes were observed in the pH, CO_2 tension, and bicarbonate concentration when a 0.6% salt solution was given. However, the chloride and total base concentrations increased slightly. Further, the decrease in the total measured acid was not as great as in the experiments where water was drunk, but since the total base did not decrease, the undetermined acid concentration was equally as great. A control specimen taken 2 hours after the short term experiment showed the same tendency as when water was drunk with the exception that the total base and chloride levels tended to return toward the control level.

On the whole the changes in the acid-base balance observed are those which one might expect to occur as a result of a rapid hyperventilation of short duration. The drinking of salt solution seems to prevent a loss of base and chloride from the serum and to better enable individuals to undergo this form of fever therapy.

7944 P

Further Studies on the Creatine Content of Heart Muscle.*

CHARLES R. LINEGAR AND VICTOR C. MYERS.

From the Departments of Biochemistry and Pathology (City Hospital), School of Medicine, Western Reserve University, Cleveland.

Creatine in the form of phosphocreatine is thought to play a significant part in muscular activity, and the functional capacity of muscle is apparently reflected by its creatine content. It is of interest to determine in what degree the retention of nitrogenous products influences the level of creatine in heart muscle and how the creatine content is also influenced by clinical and morphological

* Aided by a grant from the Josiah Macy, Jr., Foundation.

manifestations of cardiac incompetency. A study of the creatine content of heart muscle should therefore offer many interesting possibilities from the standpoint of both creatine metabolism and heart disease which are complementary to each other.

A preliminary report of our work dealing with this subject was presented before this Society in January, 1932, the first publication appearing in April, 1934.[1] The concentration of creatine in the left ventricular muscle of the human was found to exceed that of the right ventricle by about 30%. Attention was also called to certain correlations between creatine content, and age and heart weight.

This general study has been continued, special attention being given to the inclusion of cases with marked nitrogen retention and heart failure. It has been observed that in cases of marked creatinine retention without heart failure there is considerable increase not only in the creatine content of voluntary muscle but also in that of the left and right ventricle. In lobar pneumonia the creatine content of both the voluntary and left ventricular muscle was appreciably increased, due possibly to retention. Low figures for the right ventricle in several cases were suggestive of right heart failure. It was a singular fact that in cases of uremia with heart failure, the creatine content of the voluntary muscle was essentially normal, despite high figures for the blood creatinine, while the creatine concentration of the left and right ventricles was reduced, the reduction being greatest generally in the left ventricle. Somewhat similar observations were made in cases with cardiac decompensation with heart failure, although in some of these cases the percentage reduction was greatest in the right ventricle. That this is due to a greater degree of incompetence of the right than the left ventricle is suggested but not proven.

At the time we began our original study, Calhoun, Cullen, Clarke and Harrison[2] presented a very interesting paper in which they pointed out that a diminished potassium content of heart muscle was invariably present in failing ventricles and suggested that this change may have been a contributing cause of such failure. The observations we have made regarding creatine in heart failure would appear to parallel very closely those of the Vanderbilt investigators on potassium, and it may well be that they represent 2 phases of the same process. At the present time we are engaged in a further study

1 Seecof, D. P., Linegar, C. R., and Myers, V. C., *Arch. Int. Med.*, 1934, **53**, 574.

2 Calhoun, J. A., Cullen, G. E., Clarke, G., and Harrison, T. R., *J. Clin. Invest.*, 1930, **9**, 393.

with the collaboration of Mangun and Reichle[3] involving the simultaneous determination of creatine, phosphorus, and potassium, but the observations are as yet too few to warrant additional conclusions.

7945 C

Experiments on Ligation of Renal Vein.

J. A. BENDER AND J. M. HAYMAN, JR.

From the Department of Medicine, Western Reserve University, and the Medical Service, Lakeside Hospital, Cleveland, Ohio.

The effect of partial or complete obstruction of the renal vein on the secretion of urine and the histology of the kidney has been investigated repeatedly. Conflicting results reported in the literature have been reviewed by Rowntree, Fitz and Geraghty,[1] Orofino,[2] and Nicastro.[3] Little has been added to the histological description of such kidneys since Buchwald and Litten's[4] report. The outstanding feature is degeneration and atrophy of the tubules with apparently relatively normal glomeruli. Other studies have been concerned with the development of an adequate collateral circulation to maintain the functional capacity of the kidney. This is apparently much better in dogs than in either cats or rabbits, and Alesandri,[5] Rowntree, Fitz and Geraghty were able to maintain dogs in excellent condition for a time even when one kidney had been removed and the other renal vein ligated. Usually, however, even in the dog when the renal vein has been ligated a progressive atrophy of the kidney follows with decrease and finally cessation of all urinary secretion. In cats and rabbits this is the constant finding.

Orofino found that from 5 to 20 days after ligation of one renal vein in dogs, the urine from that kidney was decreased in amount, and contained lower concentrations of urea and chloride but more albumin than the urine from the normal kidney. Dicker and Demoor[6] found the volume of urine from the ligated kidney greater

[3] Myers, V. C., Mangun, G., and Reichle, H. S., unpublished observations.

[1] Rowntree, L. G., Fitz, R., and Geraghty, J. T., *Arch. Int. Med.*, 1913, **11**, 121.

[2] Orofino, A., *Ann. Ital. di Chirur.*, 1932, **11**, 924.

[3] Nicastro, G., *Il Morgagni*, 1927, **69**, 2001.

[4] Buchwald, A., and Litten, M., *Virchow's Arch.*, 1876, **66**, 145.

[5] Alesandri, quoted by Orofino.

[6] Dicker, E., and Demoor, J., *Compt. Rend. Hebdomadaires Soc. de Biol.*, 1930, **103**, 503.

(2 to 3 times) than from the normal side 6 to 8 weeks after operation, while the concentration of urea was reduced, that of chloride was increased. These experiments suggested that the effect of temporary obstruction to the renal vein and the resulting anemia of the kidney produces its greatest effect on the highly specialized epithelium of the renal tubules. It seemed that if the renal vein could be clamped for a sufficient length of time to produce a severe asphyxial injury, and then the normal circulatory channels reestablished, an opportunity should be afforded to study the urine formed after such damage as well as the pathological process of degeneration, or degeneration and repair. The development by Goldblatt[7] of an ingenious clamp which easily and accurately can be tightened and loosened seemed to afford a suitable means of temporarily obstructing the renal vein with a minimum chance of producing thrombosis.

Rabbits were used entirely. They were anesthetized with sodium pentobarbital and the left renal vein exposed through a lumbar incision. The clamp was then put on the vein, tightened, and the wound closed. After from 6 to 72 hours, the wound was reopened and the clamp loosened. After from 1 to 16 days the animal was anesthetized again, the abdomen opened, the stomach and intestinal tract removed, and then cannulae inserted in both ureters. Urine flow from the injured kidney was usually very small—frequently only a few drops could be obtained even after the intravenous injection of physiological salt solution or hypertonic sodium sulphate. In a few experiments, sufficient urine was obtained for analysis. At the end of the experiment, the kidneys were removed, weighed, and blocks fixed in formalin for histological study. All experiments in which thrombus formation in the renal vein or its branches could be detected have been discarded.

Urine was obtained in acute experiments from each of 3 rabbits after the vein had been clamped for 30 minutes. The volume per minute was greatly reduced, and the urine contained albumin and red cells. The volume was not sufficient for chemical analysis. Sufficient urine for analysis was obtained in 2 of 3 animals in which the vein had been clamped 6 hours, in 1 of 3 clamped 24 hours, in none of 8 clamped 48 hours, and in 1 of 2 clamped 72 hours. Blood was drawn from the carotid artery at the end of the urine collection. The outstanding change in the urine from the injured kidney was that in spite of the smaller volume, its composition more nearly

[7] Goldblatt, H., *et al.*, *J. Exp. Med.*, 1934, **59**, 347.

approached that of the plasma. The greatest differences were in the concentrations of chlorides and urea nitrogen, the former being higher and the latter lower than from the normal side. Estimations of creatinine were probably unreliable because of the low concentrations and the fact that no account was taken of interfering chromogenic substances in the plasma. The small volume of dilute urine is at variance with the normal rabbit, in which the greater the diuresis the nearer does the urine approach an ultrafiltrate of plasma in composition.

These findings suggest that in the injured kidney, the function of the tubule cells had been seriously impaired. The low volume of urine might be due to a diminished volume of filtrate formed, if many glomeruli were occluded, or to the reabsorption *in toto* of a large part of the filtrate by the osmotic pressure of plasma proteins in the peritubular capillaries through functionally dead tubule cells.

The histological changes seen after 30 minutes' occlusion resembled those found by Huber[8] to follow obstruction to both artery and vein for a similar length of time. There was marked hyperemia, subcapsular and peritubular hemorrhages, and swelling of the tubular epithelium. The glomeruli appeared relatively normal. Occlusion of the renal vein for from 6 to 72 hours produced histological changes most marked in the proximal convoluted tubules, less in the loop of Henle, and inconspicuous in the distal convoluted and collecting tubules. The glomeruli were as a rule remarkably well preserved. The changes were in general similar to those described by Zeckwer[9] in the cat, except that the evidences of regeneration are more marked.

There were no striking differences in the histological picture of kidneys from which urine had been obtained and those from which it had not. This simply brings out again the difficulty of interpreting functional capacity from histological appearance. In all sections, there was marked difference in the apparent involvement of different tubules. This suggests the possibility that the postglomerular circulation, including the collateral channels of Huber, may vary considerably for different tubules. It would seem proper to believe that the urine obtained was derived from the less damaged nephrons, while in spite of apparently relatively normal glomeruli, the majority of nephrons were functionless.

[8] Huber, A., *Arch. f. klin. Clin.*, 1926, **141**, 51.

[9] Zeckwer, I. T., *Am. J. Path.*, 1926, **11**, 57.

7946 C

A Synthetic Vitamin A-Free Milk Suitable for Vitamin A Studies in Very Young Puppies.

W. O. FROHRING. (Introduced by H. Goldblatt.)

Busson and Simonnet[1] have shown that newly-born puppies have a relatively low reserve of vitamin A at birth and that at the time of weaning the reserve is usually only moderately higher. These results are in agreement with my own observations (to be published). Older dogs have a still higher reserve of vitamin A in the liver. In experiments (to be published) to determine the dog's vitamin A requirements, it appeared desirable, therefore, to use newly weaned puppies in order to shorten the necessary period of depletion and especially in order to be able to make some comparison with the vitamin A requirement of growing albino rats.

In the early attempts to feed to newly weaned puppies the usual dry or semi-solid vitamin A-free diet, a rather large number of animals was lost. In the experiments here reported, a synthetic bitch's milk was used, the composition of which was like that given for the natural product by Heineman:[2]

		Composition of synthetic milk	Parts
Specific gravity	1.035		
Water	75.44	Vitamin A-free soluble casein	110
Total proteins	11.17	Crisco	95
Fat	9.57	Sucrose	30
Sugar	3.09	O. M. salt mixture	7.5
Ash	0.73	Linoleic acid	1.0
Total solids	24.56	Tap water to make	1000

The milk was produced by the use of "crisco", linoleic acid, sucrose, Osborn-Mendel salt mixture and soluble vitamin A-free casein. Tap water was added in a quantity to give the same high total solid content of bitch's milk. This mixture was homogenized at 3000 lbs. pressure, placed in hermetically sealed cans and sterilized in the same manner as evaporated milk (240°F. for 15 minutes, allowing 20 minutes for coming up time and using a sterilizer with revolving reel to keep the cans in constant motion during sterilization). A supplement of dried brewer's yeast as a source of the B complex and of irradiated baker's yeast as a source of vitamin D was added before feeding.

The synthetic vitamin A-free milk described above has been used successfully in experiments on 20 puppies. No difficulty was

[1] Busson, A., and Simonnet, H., *C. R. Soc. Biol.*, 1932, **109**, 1253.

[2] Heineman, P. G., Milk, W. B. Saunders Co., 1921.

encountered in depleting the puppies. Xerophthalmia and other characteristic signs of vitamin A deficiency were obtained. Typical weight curves are given. The weight curves (Fig. 1) and general appearance of the animals (Fig. 2) indicate that this synthetic vitamin A-free diet is satisfactory for the purpose.

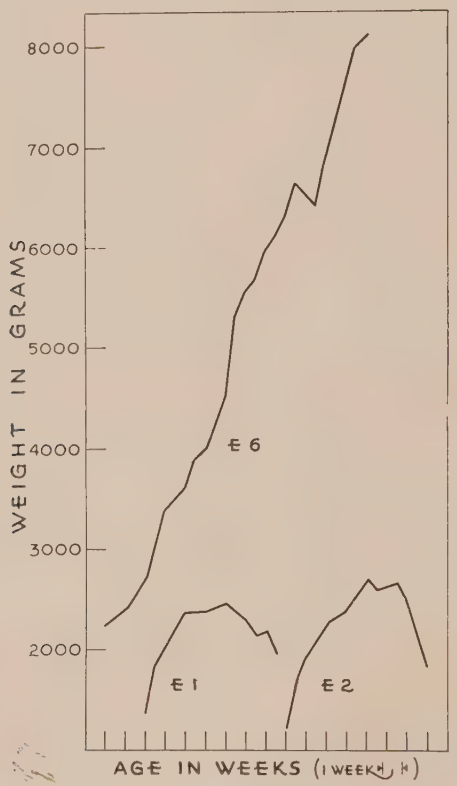


FIG. 1.

Growth curves of 3 puppies from one litter fed the vitamin A-free synthetic milk from time of weaning. E-1 and E-2 received synthetic milk only. E-6 received synthetic milk plus a daily supplement of 6300 new U.S.P.X units of vitamin A in the form of 0.3% solution of Carotene in cottonseed oil.

Two puppies have been given this milk supplemented with dried brewer's yeast, irradiated yeast and carotene in cottonseed oil for as long as 8 months.

The growth curves and general condition of those animals indicated that the milk so modified was complete for the young puppy for the first 2 or 3 months, during which time the diet consisted exclusively of this milk.

As the animals became older, additional carbohydrate was given

A B

FIG. 2.

Litter mates fed on vitamin A-free synthetic milk. "A" received synthetic milk only. "B" received synthetic milk plus 6300 new U.S.P.X units of vitamin A in the form of 0.3% solution of Carotene in cottonseed oil.

(a) (b)

FIG. 3. This is Puppy A of Fig. 2.

(a) Xerophthalmia of left eye after 10 weeks on the vitamin A-free synthetic milk.

(b) The same puppy after receiving a supplement of 2800 new U.S.P.X units of vitamin A in the form of 0.3% solution of Carotene in cottonseed oil for 13 days.

in the form of boiled polished rice to which was added an additional amount of dried brewer's yeast. Several other puppies were fed this completed milk for shorter periods with satisfactory results.

Summary. A synthetic milk has been produced that has been found satisfactory for vitamin A studies on young puppies. This milk could also serve for studies on vitamins B, G, and D.

7947 P

Preliminary Observations on the Frei Test in Lymphogranuloma Inguinale.

M. SULLIVAN AND E. E. ECKER.

From the Department of Dermatology and Syphilology and the Institute of Pathology, Western Reserve University, Cleveland, Ohio.

Since the Frei test is considered to be allergic in nature a passive transfer in the sense of Prausnitz and Küstner was attempted. Five non-tuberculous individuals who had reacted negatively to Frei antigens and one Rhesus monkey were intradermally inoculated in different skin areas of the back with 0.1 cc. Frei antigen, 0.1 cc. inactivated normal human serum, 0.1 cc. sera of typical cases of Lymphogranuloma inguinale and 0.1 cc. mixtures of normal human serum and Frei antigens. Twenty-four hours later 0.1 cc. of a potent Frei antigen was injected into the same areas but with negative results. This was repeated in 3 patients at the end of 1 and of 2 months, again with negative findings.

The Frei test as now used is often difficult to evaluate. It is evident from the reactions that the antigen far exceeds the necessary dose for a more specific and less potent reaction and that the antigens may also contain non-specific factors.

Samples of Lymphogranuloma inguinale pus were dried *in vacuo* above anhydrous $CaCl_2$, ground to a fine powder and weighed. It was found that 0.1 gm. suspended in 10 cc. of physiologic salt solution (1:100) and heated at 60°C. gives an excellent antigenic emulsion. A positive reaction was secured in one case in a dilution as high as 1:20,000.

However, the grading off usually occurred between 1:5,000-1:10,000. Therefore, the test can be performed with much more dilute antigens than are at present in use. High speed centrifugations of the antigens yielded non-reactive supernatant liquids. Berkefeld filtrates also gave negative results.

Acidification or alkalinization of the dried antigen with N/100 HCl or N/100 NaOH and neutralization prior to injection did not destroy the reacting factors. The residual lipoids from an alcohol ether extraction (3:1) were non-reactive. The protein fraction, however, gave an active antigen.

Sera from cases of Lymphogranuloma inguinale did not neutralize the Frei antigens. Recently Reiss[1] concluded that the serum of an early case of Lymphogranuloma inguinale possesses antigenic properties. However, he did not use a normal serum control. We repeated this work in a series of 20 individuals including 6 Frei positive cases with negative results in all cases of Lymphogranuloma inguinale. Our observation is in accord with the findings of Levaditi and Reinié[2] that the plasma of infected monkeys does not carry the virus but that the virus may occasionally occur in white blood corpuscles and lymph.

Fresh pus from typical cases yielding potent Frei antigens was exposed to 50% glycerol for a period of 6 weeks. Although the pus no longer produces the disease (Hellerstrom and Wassén[3]), the glycerol does not reduce its antigenic activity. This provides another means of preservation of the antigen. The use as antigens of whole pus or the separate lipoid and protein fractions failed to show complement fixation when set up with sera from patients with Lymphogranuloma inguinale.

After 48 hours sections of the skin of a positive Frei (1:100) reactor injected at the same time with (1:5) chancroidal pus showed infiltrations of lymphocytes around vascular buds, but no eosinophiles. The reaction was more marked in the case of the Frei antigen.

---

[1] Reiss, F., *Arch. Dermat. and Syph.*, 1935, **31**, 215.

[2] Levaditi, H., and Reinié, J., *Comptes rendus Soc. Biol.*, 1935, **118**, 123.

[3] Hellerstrom, S., and Wassén, E., Epidemiology and Etiology of Lymphogranuloma inguinale, Special volume, dedicated to Prof. Cantacuzène-Masson Cie, 1934.

Iowa Section

State University of Iowa, February 19, 1935.

7948 C

Effect of Heat and Alcohol Extraction on the Nutritive Value of Casein.

H. W. SCHULTZ, W. H. SEEGERS AND H. A. MATTILL.

From the Biochemical Laboratory, State University of Iowa, Iowa City.

The methods employed for the purification of casein for experimental diets require either the use of solvents to extract extraneous materials or the application of heat to destroy the unwanted accessories, or both solvents and heat may be employed. Casein treated in either or both of these ways does not permit optimum growth in experimental animals. The experiments described below, which are, in many respects, similar to those reported from other laboratories, can readily be explained on the basis of the removal of a nutritional factor, with or without the coincident impairment of the protein itself.

An attempt was first made to demonstrate what effect, if any, alcohol extraction of casein might have on the value of a diet in supporting lactation in rats in the first and second generations. By the method of Kozlowska, McCay and Maynard,[1] lactation was studied on stock females which were fed the following diet beginning 2 days after parturition: protein, 18%; hydrogenated cottonseed oil (crisco), 22%; corn starch, 41.5%; salts,[2] 4.5%; agar agar, 2%; yeast,* 10%; cod liver oil, 2%. The protein was commercial casein (diet A) or casein which had been extracted in a percolator for 4 days with boiling 95% alcohol (diet B). This same method of extraction, first described by Sperry, was used for all preparations. At the end of the 40-day lactation period the young

[1] Kozlowska, M., McCay, C. M., and Maynard, L. A., *J. Nutr.*, 1932, **5**, 61.

[2] Hawk, P. B., and Oser, B. L., *Science*, 1931, **74**, 369.

* Kindly supplied by Northwestern Yeast Company.

from these litters were continued on the same diet and their growth rate (Fig. 1) and lactation capacity were also studied.

From Table I it can be seen that lactation was much better on diet A than on diet B, but this difference was only slightly accentuated in the second generation. The growth rate of the second generation males was inferior on the extracted casein but the females did not show this difference (Fig. 1). Such sex differences are not uncommon in nutrition studies and their cause is obscure. Additional male rats from inbred stock ("standard" animals) again revealed the same difference in the growth-promoting capacity of the 2 rations (Fig. 1).

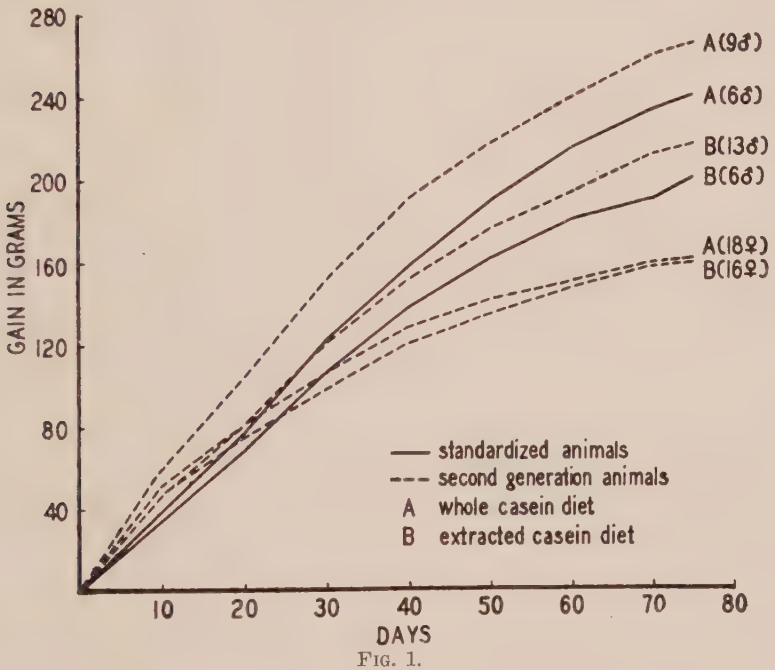


FIG. 1.
Effect of Alcohol Extraction of Casein on its Nutritive Value for Growth.

TABLE I.
Effect of Extraction of Casein on Lactation.

Diet	No. of litters	Litters reared	Whole litters reared	Surviving young %
		First generation (40 days)		
A	8	6 (75%)	5 (63%)	73
B	13	6 (46%)	4 (31%)	40
		Second generation (15 days)		
A	12	8 (67%)	6 (50%)	64
B	11	4 (36%)	2 (18%)	24

For studies on the effect of heat and on the corrective influence of various extracts the animals used were equally divided as to sex and weighed from 130 to 140 gm. The yeast and cod liver oil of the basal ration were replaced by starch and daily supplements of 0.5 gm. yeast and 5 drops of cod liver oil were fed. Casein extracted for 4 days and heated for 2 hours at 120°C. gave the least satisfactory growth of any of the preparations (Table II); prolonged heating seems to destroy or alter more effectively than short heating, even at a higher temperature.

TABLE II.

Effect of Alcohol Extract of Wheat Germ as Supplement to a Diet Containing Extracted and Heated Casein on the Growth of Rats.

No. of lot	No. of animals	Treatment of casein	Addition	Gain in wt. gm.	Food intake gm.	Gain per gm. of protein gm.
1	6	Extracted, heated 150°C., 30 min.	None	41.9	168.0	1.386
2	6	Same	Hot alcohol extract of wheat germ	42.1	159.2	1.469
3	5	Same, 120°C., 2 hrs.	None	27.6	159.0	0.964
4	6	Same	Hot alcohol extract of wheat germ	40.6	149.4	1.510

This poorly utilized ration was improved by supplementing with a hot alcohol extract of wheat germ. (Compare lots 3 and 4, Table II.) The supplementary effect of such an extract was practically without significance when fed with a diet containing extracted casein heated for 30 minutes at 150°C. (Lots 1 and 2.)

Ether extracts of wheat germ were ineffective. The improved growth which Coward and her coworkers[3] obtained by supplementing their basal diet with similar extracts may have been due to traces of vitamin A in the oil, since the animals were on an A-deficient diet. Their favorable results with ether extracts of wheat germ and alcohol extracts of wheat germ and casein are therefore not conclusive proof of a new dietary factor. The foregoing experiments, however, show that the presence of such a factor in casein and wheat germ can be demonstrated even when all other known dietary essentials are amply supplied.

The favorable effect of added extracts in increasing growth rate

[3] Coward, K. H., Key, K. M., and Morgan, B. G. E., *Biochem. J.*, 1929, **23**, 695.

does not preclude the possibility of protein damage. Morgan[4] and Fixsen and Jackson[5] have demonstrated that heating lowers the biological value of casein, but the nature and extent of such damage, under given conditions, are not clear. The possibility of a change in the digestibility of the protein, similar to that found with animal tissues (Seegers[6]), is not excluded. The supplementary value of alcohol extracts of wheat germ is less well explained by such considerations than on the generally accepted grounds that such extracts provide a labile factor (B_4?)[7, 8] which is removed from casein by alcohol extraction and which is present in only small amounts if at all in yeast.

7949 P

Tyrosinase in Ontogenesis (Orthoptera)

J. H. BODINE AND E. J. BOELL.

From the Zoological Laboratories, State University of Iowa.

Variations in concentration of tyrosinase throughout the entire embryonic development of the grasshopper, *Melanoplus differentialis*, have been determined by measuring the oxygen uptake of the tyrosinase-tyrosine reaction with the Barcroft-Warburg apparatus. Tyrosinase activity of eggs at different developmental stages has thus been expressed as the amount of O_2 consumed per 100-minute interval at 25°C. in the oxidation of a given amount of tyrosine by the enzyme extracted (in phosphate buffer pH 8.0) from 20 eggs.

The growth curve for tyrosinase in the whole egg is sigmoid during the first 3 weeks. Maximum enzyme concentration is reached on the 20th day and is maintained at this level throughout a period of suspended embryonic development (diapause) which occurs then. The post-diapause developmental period, during which the embryo pigments and hatches, is characterized by a decrease in concentration of tyrosinase.

The largest part of the tyrosinase content of the egg is found in the yolk and in the serosa cells and fluids surrounding the embryo. The amount of tyrosinase in the embryo alone is low but increases

[4] Morgan, A. F., *J. Biol. Chem.*, 1931, **90**, 771.

[5] Fixsen, M. A. B., and Jackson, H. M., *Biochem. J.*, 1932, **26**, 1923.

[6] Seegers, W. H., in press.

[7] Reader, V., *Biochem. J.*, 1929, **23**, 689.

[8] Halliday, N., *J. Biol. Chem.*, 1934, **106**, 29.

during growth. During post-diapause development the embryo engulfs yolk and serosa cells so that it eventually contains most of the enzyme rich egg components. Apparently a gradual transfer of enzyme from yolk to embryo then occurs. The amount of tyrosinase in the egg membranes is low and remains practically static during the whole of development.

Attempts to obtain the enzyme from nymphs (after the 3rd instar) and from adults have been unsuccessful.

7950 C

Effect of Skim-milk, Lactose, Vinegar and Iodine on the Quantitative Character of a Coccidian Infection.*

E. R. BECKER AND N. F. MOREHOUSE.

From Iowa State College.

Skim-milk and lactose supplements in the diet were recommended by Beach and Davis[1] as affording a considerable degree of protection against coccidiosis in poultry. The explanation of the claimed benefits was the production of an abnormal degree of acidity in the caeca of the birds, which in turn injured or destroyed the sporozoite or merozoite stages of the parasites. Since the rat is a favorable host for the study of a coccidian infection, an experiment was planned in which one series of hosts received the regular growing ration made up to 40% with skim-milk beginning 4 days before the date of the first infection and continuing throughout the experiment, and another series received its regular ration without skim-milk. Infection was accomplished by forced feeding of from 1,500 to 3,000 oocysts of *Eimeria miyairii* daily for 4 or 5 successive days. The counts of the oocysts eliminated in the fecal pellets were taken as the index of the infection intensity. Seventeen rats on the skim-milk diet eliminated from 94 to 376 million oocysts each; mean, 203.24 millions. Sixteen controls on the regular diet eliminated from 72 to 464 million oocysts each; mean, 237.25 millions. The difference in the means divided by the standard deviation of the difference ($34.01 \div \sqrt{737.25 + 381.334}$) was 1.02, a nonsignificant value. Through the kindness of Dr. Donald Starr of the Chemistry Department it was possible to determine the

* Project No. 40, Industrial Science Research Fund, Iowa State College.

[1] Beach, J. R., and Davis, D. E., *Hilgardia*, 1925, **1**, 167.

pH of the entire content of the small intestine of some of the rats at the end of the experiment. The values for 3 rats on the milk diet were 6.92, 6.74, and 7.28; for 3 controls, 6.60, 6.75, and 6.48. It is evident that the skim-milk diet did not produce an abnormally acid condition in the small intestine of the rat, and that the parasite population was not reduced by the diet. Another similar experiment in which the percentage of skim-milk was reduced to 22% and the doses of parasites were lighter gave mean yields of 123 million oocysts for 8 rats on the special diet and 122 million oocysts for 7 on the regular diet.

An experiment was carried out exactly as the former except that the diet was made up to 20% with lactose instead of adding skim-milk supplement. Four rats receiving the lactose eliminated a mean of 211 million oocysts, while the 4 controls eliminated a mean of 207 million oocysts.

Kerr and Botham[2] announced that they had found iodine of value in the control and treatment of avian coccidiosis. The infected birds were given to drink only a 1:8 mixture of (a) a stock solution composed of 1 gm. of resublimated iodine, 2 gm. of potassium iodide, and 50 cc. of water added to 450 cc. of milk and (b) water.

Allen[3] found that ordinary vinegar added to the drinking water of fowls in the proportion of 1 to 79 reduced the numbers and vitality of the oocysts eliminated. To test out these measures in a preliminary way, 3 rats out of a litter of 10 were given to drink only the Kerr and Botham mixture, 3 the diluted vinegar, and 4 tap water. The day after they were put on these drinks each rat received 3,000 infective sporulated oocysts, and the same dose each of the succeeding 4 days. The counts (in millions) for the recipients of iodine were as follows: 525, 436, 432. The same for the recipients of vinegar: 409, 645, 449. The controls: 517, 346, 370, 413. This test experiment seems to indicate that iodine and vinegar have no general value as coccidicidal agents.

In conclusion, it has been shown that skim-milk or lactose supplement in the diet, iodine in the drinking water, or vinegar in the drinking water do not exert any restraining influence upon the numerical increase of the coccidian population in the alimentary tract of the mammal.

---

[2] Kerr, W. R., and Botham, G. H., *Vet. J.*, 1931, **87**, 10.

[3] Allen, E. A., *Poultry Science*, 1933, **12**, 324.

7951 P

Characteristics of Sporulating Facultative Bacteria Producing Gas from Lactose.

ROGER PORTER, C. S. McCLESKEY AND MAX LEVINE.

From the Department of Bacteriology, Iowa State College, Ames, Iowa.

Facultative spore-forming bacteria, fermenting lactose with gas production, have been encountered on a number of occasions in water supplies. As these bacteria are gram negative and frequently do not show spores on carbohydrate media, they have been a source of confusion in the interpretation of bacterial water analysis for the incidence of members of the colon group. Such organisms, which were isolated for the first time in Iowa from the chlorinated drinking water at Iowa City in 1921 by Hinman and Levine, were thought to be rare; but evidence is accumulating to indicate that they are quite ubiquitous, having since been isolated from soil, animal and human feces, decaying and canned vegetables, sewage, grains, and eggs.

There is considerable confusion in the literature concerning the identity and differentiation of species allocated to this group of bacteria. This report presents, briefly, a study of all strains described in the literature, which are available, together with a number of freshly isolated cultures.

The study included 63 freshly isolated strains and authentic cultures of all described strains except 2 which apparently are extinct.

There was considerable variation in the size of the bacteria on different culture media. In a few cases terminal oval spores were seen within the sporangia, but generally they were observed free. All strains were actively motile.

Culturally, the strains showed very little difference. On nutrient agar at 37°C., growth was slight, effuse, and transparent. On sugar-containing agar, growth was spreading and slimy. Growth in nutrient broth was slight and with some sediment; in sugar broth the medium frequently became very viscous.

Colony characteristics vary with the medium. On nutrient agar, well isolated colonies are small and transparent. On sugar-containing media, colonies are extremely variable, but are transparent and usually very slimy. On Endo's medium, colonies are pink to red in color and may be small and round or large and amoeboid. Development of a sheen and red halo around the colonies were not constant characteristics. On eosin methylene blue agar, growth

was markedly retarded; small pin-head colonies appeared after 48 to 72 hours; the production of a sheen was variable.

On the basis of the physiological tests employed, the organisms fall into 2 groups (Table I) which may be differentiated into a (V.P.—) group, which grew well at 45°C. and fermented sorbitol and rhamnose with acid and gas production; and a (V.P.+) group which did not grow at 45°C. and did not produce acid or gas from sorbitol and rhamnose. These subdivisions will be referred to as the macerans (V.P.—) and the polymyxa (V.P.+) groups, respectively.

TABLE I.
Differential Characteristics in Facultative, Sporulating, Aerogenic Bacilli.

	Aero. macerans group	*Aero. polymyxa* group
No. of Strains	16	71
Character	% Positive Reactions	
Growth at 45°C. (48 hr.)	100	0
Growth at 13°C. (1 week)	0	100
Acid and gas in Sorbitol (72 hr. 37°C)	100	0
Acid and gas in Rhamnose (48 hr. 37°C.)	100	0
Voges-Proskauer reaction (72 hr. 37°C.)	0	100
Gelatin liquefaction (96 hr. 37°C.)	0	88.8
Milk coagulated (72 hr. 37°C.)	0	84.5
Agglutinated by *Aero. macerans* serum (2 sera tested)	100	0
Agglutinated by *Aero. polymyxa* serum (9 sera tested)	0	*

* The 9 sera show that the group is very heterogeneous serologically. Each tested serum agglutinated its specific organism and a number of other strains, but no one serum agglutinated the entire group.

Indol and H_2S were not produced; citric and malonic acids were not utilized as a sole source of carbon by any of the strains; nitrates were reduced to nitrites by all cultures.

The organisms were tested in 33 carbohydrates and polyatomic alcohols. Most of these were fermented with acid and gas production; however, only rhamnose and sorbitol seemed to be of any differential value.

Serologically, the 2 groups appear to be distinctly different. The macerans group is an antigenic entity; the polymyxa group, however, shows a great deal of heterogeneity and further work is in progress. The strains tested were not pathogenic for rabbits.

The results indicate that there exist 2 distinct groups among the facultative, sporulating aerogenic bacilli. The 2 groups are strikingly different, physiologically and serologically.

If the genus *Aerobacillus* Donker (1926) is to be adopted, the following species are recognized for the present:

1. *Aerobacillus polymyxa* (Prazmowski) Donker 1926, syn:
   Clostridium polymyxa Prazmowski 1880
   Granulobacter polymyxa Beijerinck 1893
   Bacillus polymyxa Beijerinck and Den Dooren De Jong 1923
   Astasia asterospora Meyer 1892
   Bacillus asterosporus (Meyer) Migula 1900
   Bacillus mycoides var. ovoaethylicus Wagner 1916
   Bacillus aerosporus Greer 1928.
2. *Aerobacillus macerans* (Schardinger) Donker 1926, syn:
   Bacillus macerans Schardinger 1905
   Bacillus acetoethylicum Northrop 1919
   Aerobacillus acetoethylicus (Northrop) Donker 1926.

7952 C

Ligation of Carotid and Vertebral Arteries in Monkeys.

M. D. GARDNER.* (Introduced by J. A. Greene.)

From the Department of Surgery, University of Iowa.

The mortality resulting from ligation of the common carotid artery in man varies from 7 to 90%, depending on the condition for which the ligation was done, and the age of the patient.[1] The value of pre-ligation compression of the carotid artery to establish collateral circulation, is not definitely established. This work was primarily undertaken to determine the effect of pre-ligation compression of the common carotid in monkeys. Dogs and rabbits seldom develop symptoms after ligation of a common carotid artery,[2] whereas we hoped that monkeys, whose cranial blood supply is quite similar to that of man,[3] would react in a way comparable to man. However, we were unable to cause paralysis or death in monkeys by ligation of a common carotid artery, or by simultaneous unilateral ligation of vertebral, internal, external and common carotid arteries. The ligations were continued in an effort to determine

* I wish to express sincere appreciation to Dr. C. G. Barer, Instructor in Neurology, whose collaboration in the histologic study was invaluable.

1 Freeman, L., *Ann. Surg.*, 1921, **74**, 316.

2 Sussi, L., *Ann. ital di chir.*, 1932, **11**, 311.

3 Hartman and Straus, Anatomy of the Rhesus Monkey, Chapter XII, p. 251.

TABLE I.

Exp.	Procedure Ligation of	Symptoms and Results	Gross and microscopic pathology. (Positive findings only)
1—A.	Rt. Com. Carotid	No change 6 days	
B.	Rt. Int. Carotid	No change 6 days	
C.	Rt. Thyrocervical axis	No change 24 days	
D.	Rt. Vertebral	Rt. foreleg paralyzed (1) Death in 6 days hemorrhage stump of Vertebral	(2) Perivascular cellular infiltration, bilateral.
2—A.	Rt. Vertebral Int. Ext. and Com. Carotids	No change in 11 months	
B.	Lt. Com. Carotid	Animal comatose, no paralysis. Death in 2 days.	Cerebellum showed glial proliferation, absence of Purkinje cells and of normal markings. Patchy areas of early necrosis in cerebrum. (3)
3—A.	Rt. Vertebral, Thyrocervical axis, Int. Ext. and Com. Carotids	Death from pneumonia in 2 days. No symptoms of cerebral ischemia.	Hemorrhage bilateral in area of Int. capsule. Sections cerebellum normal. Cerebrum showed congestion of blood vessels, perivascular hemorrhage and cellular infiltration.
4—A.	Rt. Vertebral Int. Ext. and Com. Carotids	No change in one year.	
B.	Lt. Com. Carotid (4)	Slight ataxia and weakness right foreleg. No paralysis. Stupor in 2 hr. Death in 4 hr.	No change. (5)
5—A.	Rt. Vertebral, Int. Ext. and Com. Carotid	Transient vertigo and ataxia; otherwise no change in 11 months	
B.	Lt. Com. Carotid	No change in ½ hr. Killed by inject. ether	Microscopic shows (7,8) blanching of tissues about vessels.
6—A.	Lt. Vertebral Thyrocervical axis, Int. Ext. and Com. Carotids	Ataxia. Animal fell to right. Rt. side weak and Rt. extremities moved poorly. Symptoms decreased gradually. Death 6th post-operative day. Definite pneumonia.	Some softening Ft. frontal lobes. (9) Tissue edematous and vacuolated. Mid and ant. portions of cerebrum took a hemogeneous stain. Ganglion and glial cells pale staining and nuclei indistinct. Many cavities. Phagocytic cells about larger vessels on Rt. Some perivascular hemorrhage.

(1) Due to traction on brachial plexus?
(2) Injection of ink into Lt. Common Carotid. Vessels of both sides filled equally well.
(3) Rt. frontal lobes least involved.
(4) Local anesthesia.
(5) Definite intimal thickening of arteriosclerosis.
(6) Lt. Common Carotid first digitally compressed for one hour. No change except for periods of excitement, hyperpnea and nystagmus.
(7) Barium in formaldehyde allowed to flow into the cannulized Lt. Common Carotid. Vessels of both sides equally well filled as shown by X-ray and postmortem.
(8) Due to ether injection? Similar in amount on both sides.
(9) Postmortem had been delayed 24 hours.

how much alteration of cranial blood supply the monkey would tolerate, and what changes in the brain were produced.

Table I summarizes the procedures and results obtained.

1. The rhesus monkey is unaffected by ligation of a common carotid artery, and in more than one-half of the procedures, will be unaffected by simultaneous ligation of the common, internal and external carotid and vertebral arteries. In no instance did hemiplegia result; in one there was weakness of the opposite side; and in 2, transient ataxia

2. If, in addition to the ligation of the main cervical vessels on one side, the common carotid artery on the other side is ligated, the animals will die, even though the second ligation is done 9 months to a year after the first. The survival of the animals following the first ligation is due to the adequate blood supply obtained from the vessels of the opposite side, as demonstrated by the injection of ink, or of barium solution into the common carotid of one side, and its equal distribution in the superficial vessels of both hemispheres. Little collateral develops on the side undergoing primary ligation of the main vessels, as death occurs when the remaining common carotid is ligated.

3. Of the 6 animals undergoing ligation of the main arteries of one side, only one showed gross brain changes, (Exp. 6) and these were probably post-mortem, as necropsy had been delayed. On the same basis the microscopic changes here cannot be accepted as due to cerebral ischemia alone. The pathology in the cerebellum in Experiment 2 represents an older change than would be expected to occur in the 2 days intervening between the second ligation and death, and is, therefore, probably the result of the primary ligations. The areas of early necrosis in the cerebrum in this instance are undoubtedly the response to the ligation of the left common carotid. There were no gross or microscopic changes in the brain of the other animal dying from the ligation of the remaining carotid. (Exp. 4). This may be due to the short period (4 hours) between the ligation and death. We may conclude that animals may die of cerebral ischemia, yet have no gross or microscopic change in the brain.

4. The monkey, like the dog and rabbit, does not react to ligation of a common carotid artery in a way comparable to man, and is not a satisfactory animal for pre-ligation compression experiments.

7953 P

Reducing Power of Hemolymph from the Roach, Periplaneta americana Linn., with Special Reference to Coagulation.

J. FRANKLIN YEAGER AND R. W. FAY.

From the Department of Zoology and Entomology, Iowa State College.

Hemolymph (blood) coagulation of the roach, *P. americana,* involves cytolysis of the hemolymph (blood) cells. This paper reports experimental results bearing on the question: do reducing substances enter the plasma from the coagulating cells? The Hagedorn-Jensen blood sugar micromethod[1] was used to determine total reducing power, expressed as mg. glucose per 100 cc. hemolymph. Hemolymph samples were obtained from severed antennae, a single sample coming from a single animal. Ten samples were from animals submerged in water at 60° for 10 minutes to fix the cells, 10 from animals subjected to glacial acetic acid vapor until cell fixation had occurred and 10 samples of "serum" were obtained from untreated animals by collecting the normal hemolymph under oil to prevent drying, letting stand 10-15 minutes to allow cell coagulation to occur and then removing the uncoagulated fluid with a fine glass capillary tube. The "serum" was transferred from the capillary tube to a 0.1 cc. micropipette, graduated to 0.001 cc., used also to measure the volumes of samples of uncoagulated hemolymph. All of the samples were introduced from this pipette into the tube for precipitation of cells and proteins, as required by the method.

All of the animals treated with heat and acid to inhibit coagulation and half of the untreated animals were imagos; the others were large nymphs. The mean total reducing powers and their standard deviations are 63.2 ± 10.2 for the heat-treated imagos (whole blood), 57.3 ± 15.7 for the acid-treated imagos (whole blood), 65.5 ± 20.5 for the untreated imagos ("serum"), 65.3 ± 9.1 for the untreated nymphs ("serum"), 65.4 ± 16.3 for the untreated animals and 62.0 ± 14.4 mg. "glucose" per 100 cc. hemolymph for the whole group of 30 animals.

These results indicate that (1) the mean total reducing power, expressed in terms of glucose, of the hemolymph plasma of this roach is of the order of 62.0 mg. "glucose" per 100 cc. This is probably greater than the true "blood sugar" value. (2) The total reducing power of the hemolymph plasma is not significantly altered

[1] Peters, J. D., and Van Slyke, D. D., "Quantitative Clinical Chemistry," Vol. II. Methods, 1932.

by (a) fixation of the hemolymph cells with heat or acetic acid treatments or (b) the occurrence of cell coagulation; this implies that no quantities of reducing substances, detectable by the method used, pass from cytolysing hemolymph cells to plasma during the 10-15 minute coagulation period following sampling. (3) The total reducing powers of imaginal and large nymphal hemolymph plasmas are essentially the same.

Pacific Coast Section

7954 C

Effect of Administered Glucose upon Amino Nitrogen Content of the Blood.*

JAMES MURRAY LUCK, BURT LINCOLN DAVIS, JR., AND WALTON VAN WINKLE, JR.

From the Biochemical Laboratory, Stanford University, California.

The experiments here recorded arose from investigations into the influence of adrenalin on the amino nitrogen content of the blood. It was recently shown by Davis and Van Winkle[1] that the amino-acid-lowering property of insulin, which we have been studying for some years, is directly due to adrenalin, secreted in response to the administered insulin. Part of the proof is based upon the observation that insulin is without effect on the blood amino acids in adrenodemedullated rabbits. However, the administration of adrenalin induces in these, as well as in normal animals, the characteristic lowering.

Since the response seems to be due specifically to adrenalin, it is possible to infer, under appropriate conditions, that a lowering in blood amino acids is indicative of a discharge of this hormone. We have now made use of this hypothesis to test the response of the adrenal medulla to administered glucose.

To 15 normal students (13 males, 2 females) from 16 to 30 years of age, who volunteered as subjects, glucose was administered after fasting periods of 15 hours. To 4 others, who served as controls, water was given in quantities comparable to those received with the glucose by the experimental subjects. Blood sugar (Folin) and amino nitrogen (Danielson) determinations were made at zero, one, 2 and 4 hours after the first administration of glucose. Glucose was given at zero, one and 2 hours. The results are summarized in Table I.

* This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

1 Davis, B. L., Jr., and Van Winkle, W., Jr., *J. Biol. Chem.*, 1934, **104**, 207.

TABLE I.
Hypoaminoacidemia induced by glucose.

Hrs. after commencement		Glucose administered, gm.	Blood-sugar content, % of initial value		Amino N content, % of initial value		Subjects
			Range	Aver.	Range	Aver.	
0		100	100 (98-110)§	100 (104)§	100 (7.0-8.1)§	100 (7.6)§	
1		50					10
2		25	83-113	92	69-84	77	
4			72-105	91	66-77	70	
0		50	100	100	100	100	
1		25	83-134	100	83-101	89	
2		25	79-104	91	83- 93	88	5
4			83-98	89	77- 93	87	
0			100	100	100	100	
1	Controls		100-106	103	93-103	98	
2	No glucose		97-106	102	99-104	103	4
4			103-104	104	96-102	99	

§ Initial absolute values are given in parenthesis.

As a simple explanation of these observations we suggest the following mechanism. Insulin is first secreted in response to the administered glucose. This is indicated by the absence of sustained hyperglycemia,† the evident trend towards hypoglycemia after one or 2 hours, and is in accord with the now abundant evidence of many investigators. The secreted insulin then evokes a discharge of adrenalin, the agent immediately responsible for lowering the amino acid content of the blood. The evidence pertaining to the discharge of adrenalin in response to insulin is presented in numerous papers cited in our earlier report.[1] Since the reduction in amino acid content is not attended by a low blood sugar, we conclude that insulin, *per se*, in the absence of appreciable hypoglycemia is able to stimulate the adrenal medulla.‡ We propose, eventually, to

† In 2 instances, several blood-sugar determinations were made during the first hour after the initial dose of glucose was given; in confirmation of many similar observations recorded in the literature, there was a very marked but transitory hyperglycemia.

‡ It is pertinent to mention that in the course of other studies by Daniels and Luck (hitherto unpublished) glucose was given as in the experiments recorded here. The total quantities administered were larger and were divided into 5 doses, administered over 4-hour periods. The initial hyperglycemia was more prolonged, the amino acid concentration fell, and so also did the blood inorganic phosphorus. The phosphate decrease, which adrenalin is able to induce, is itself indicative of adreno-medullary stimulation and supports the conclusions drawn from our amino acid studies.

test this hypothesis by the administration of glucose to adrenalectomized animals.

7955 C

Stability of Streptofibrinolysin.*

R. R. MADISON AND J. K. VAN DEVENTER. (Introduced by W. H. Manwaring.)

From the Laboratory of Bacteriology and Experimental Pathology, Stanford University, California.

Quantitative studies of antifibrinolytic immunity are made difficult by the multiplicity of reacting (or conditioning) factors in sterile† streptococcus filtrates and by the non-applicability of simple

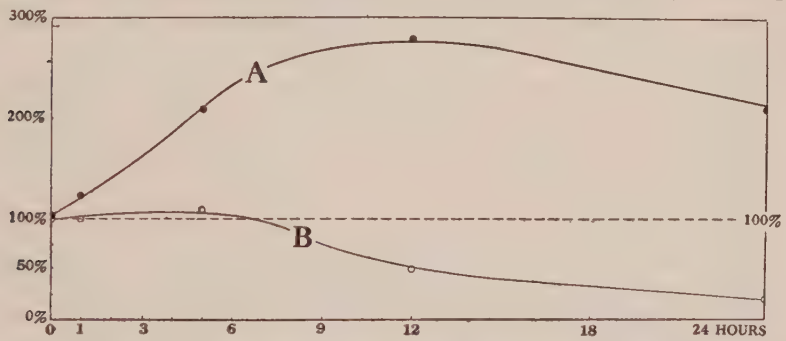


FIG. 1. *Serological Exaltation of Fibrinolytic Titer.*

Chamberland (L-3) filtrates from 24-hour broth cultures of *S. hemolyticus* were diluted with an equal volume of 0.25% to 0.5% normal horse serum, control dilutions being made with the same volume of 0.8% NaCl-solution. The dilute filtrates were then incubated at 37°C. At various times during this incubation, samples were titrated for their antihuman fibrinolytic function.

The lytic unit selected for these titrations was the minimum volume of the original filtrate that would cause demonstrable lysis of the serum-free human-fibrin clot, by the end of one hour, materials, dilutions, etc., being identical with those used by Tillett and Garner.[1] The original titer of the filtrate is recorded as 100%.

A. 50% Streptococcus filtrate A containing 1:600 normal horse serum. (Composite data from two titrations.)

B. Control test with serum-free filtrate.

* Supported in part by the Eli Lilly and Co. Streptococcus Research Fellowship of Stanford University and in part by the Rockefeller Fluid Research Fund of Stanford Medical School.

† These filtrates gave no demonstrable growth in veal-infusion broth or on routine 5% rabbit-blood agar.

1 Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485. Van Deventer, J. K., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 366.

physicochemical laws to the neutralization of such filtrates with streptococcus antiserum.

A 200% increase in effective fibrinolytic titer often takes place in control mixtures of streptococcus filtrate and normal horse serum. A typical example of this serological exaltation of fibrinolytic titer is recorded in Fig. 1.

Similar activations, depolymerizations, maturizations or apparent proliferations of the fibrinolysin take place during the process of neutralization with specific immune serum.‡ With border-line serum doses the resulting diphasic or triphasic neutralization curves give data, from which the titer of the antiserum is difficult to calculate. Typical curves of this type are recorded in Fig. 2.

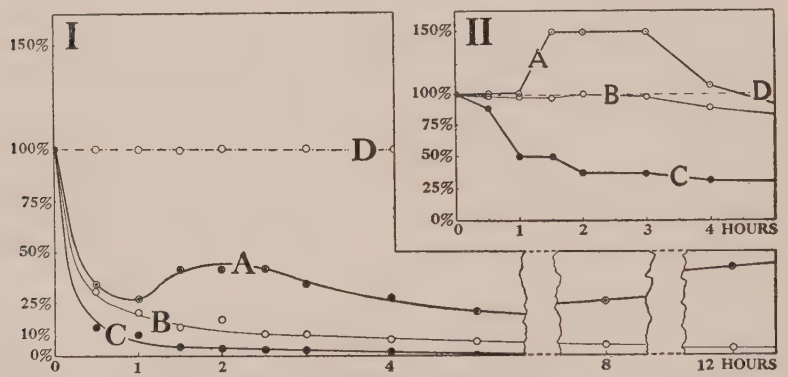


FIG. 2. *Neutralization of Streptofibrinolysin with Anti-streptococcus Serum.*

Titration technic as in Fig. 1, but with the substitution of specific immune serum in place of normal horse serum.

I. Neutralization of streptococcus filtrate C with commercial antiserum X. A, diphasic curve with 1:3,000 antiserum; B, monophasic curve with 1:2,000 immune serum; C, 1:1,000 immune serum; D, control test with 1:2,000 normal horse serum.

II. Neutralization of streptococcus filtrate D with commercial antiserum Y. A, quasi-proliferation of the fibrinolysin in the presence of 1:6,000 immune serum; B, apparent non-neutralization of the lysin with 1:3,000 immune serum; C, typical neutralization with 1:1,000 immune serum; D, control test with 1:1,000 normal horse serum.

The fibrinolytic titer of a streptococcus filtrate is often inadvertently increased under routine experimental conditions, even in the absence of normal or specific immune serum. Such quasi-proliferation of the fibrinolysin is quite constant, for example, in dilute filtrates stored at refrigerator temperatures. Typical data are recorded in Fig. 3.

‡ The normal and specific immune horse serums used in these tests were kindly furnished by Eli Lilly and Co., The Cutter Laboratory, Lederle Laboratories, E. R. Squibb and Sons, and Parke, Davis and Co.

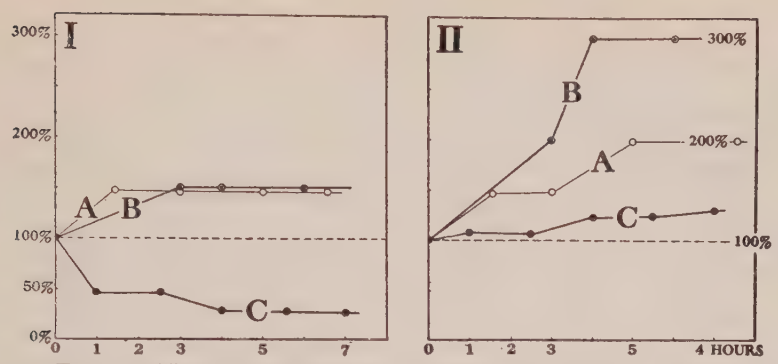


FIG. 3. *Effect of Refrigeration on Dilute Streptococcus Filtrate.*

Filtrates A and B were diluted with 4-volumes and filtrate C with 14-volumes of 0.8% NaCl-solution.

I. Change in lytic titer in dilute filtrates A, B and C as a result of storage at 37°C.

II. Parallel changes in titer of control samples stored at 4°C.

No theory is as yet suggested as to the probable mechanism of these unprecedented increases in lytic titer.

Whether or not similar augmentations of lytic activity take place in the animal body (*e. g.*, as a result of the administration of subtherapeutic doses of antistreptococcus serum), is a problem of practical clinical interest.

7956 C

Effects of 2-4 Dinitrophenol on Respiration of Commercial Cake Yeast.*

J. FIELD, 2ND., A. W. MARTIN AND S. M. FIELD.

From the Department of Physiology, Stanford University.

De Meio and Barron[1] state that neither their findings nor those of Ehrenfest and Ronzoni[2] support "Field, Martin and Field's[3, 4]

* Supported in part by a grant from the Rockefeller Fluid Research Fund of the Stanford University School of Medicine.

1 De Meio, R. H., and Barron, E. S. G., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 36.

2 Ehrenfest, E., and Ronzoni, E., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 318.

3 Field, J., 2nd, Martin, A. W., and Field, S. M., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 56.

4 Field, J., 2nd, Martin, A. W., and Field, S. M., *J. Cell. and Comp. Physiol.*, 1934, **4**, 405.

contention that dinitrophenol is active toward yeast only in its undissociated form." Bearing in mind the fact that our "contention" relates only to studies on a pure culture of yeast, let us see to what extent the published data cited support the statement of De Meio and Barron.

Determination of the optimal concentration of 2-4 dinitrophenol (hereinafter DNP) at more than one pH level is essential for the decision of the point at issue. Ehrenfest and Ronzoni reported an optimal concentration of 0.36 mg. % DNP at pH 4.5 for their yeast. De Meio and Barron also reported work on yeast at one pH level only, viz: 6.64, and failed to state whether or not their dosage was optimal. Hence the published data cited afford no basis for the statement of De Meio and Barron, in fact the concentration of undissociated DNP corresponding to the optimum found by Ehrenfest and Ronzoni (assuming use of the sodium salt) is 0.00399 millimolar, which agrees very well with the values reported in the present paper for commercial yeast at other pH levels.

Moreover, De Meio and Barron used commercial yeast suspensions, which always contain several strains of yeast as well as numerous bacilli and cocci. Van Niel and Visser 'T Hooft[5] have pointed out that pure cultures are essential for reliable work in microbiology. Conclusions drawn from experiments on heterogeneous populations cannot safely be regarded as generalizations, although it may chance that a conclusion drawn from work on pure culture will also apply in the presence of other species. The experiments reported below are a case in point, and show conclusively that undissociated DNP is the active agent in the stimulation or inhibition of yeast respiration in commercial yeast suspensions as well as in suspensions of pure culture.[3, 4]

The experimental procedure was the same as in our previous work,[4] except that commercial cake yeast was used instead of pure culture. A small piece of yeast cake was fragmented, washed by centrifugation in glucose-phosphate, and finally suspended in glucose phosphate solution at the desired pH. Heavy bacterial contamination was always present in spite of repeated washing.

The most important link in the chain of evidence showing that undissociated DNP is the active agent in the stimulation of yeast respiration is the finding that in doses evoking optimal stimulation the concentration of the undissociated form is quite constant over a wide pH range, while the total concentration causing optimal stim-

[5] Van Niel, C. B., and Visser 'T Hooft, F., *Ber der Deutsch. Chem. Gesell.*, 1925, **58**, 1606.

ulation is a function of pH. A series of 14 experiments showed that this is true for contaminated commercial yeast suspensions within quite narrow limits. At pH 6.8 the total optimal concentration of sodium dinitrophenoxide was 60 mg. %, while at pH 5.2 it was 2.5 mg. %, a 24-fold difference. The corresponding concentrations of undissociated DNP are 0.00444 and 0.00692 millimolar respectively, which is good agreement in view of the unsatisfactory nature of the experimental material.

If undissociated DNP is the active agent, a given total concentration of the drug should cause stimulation at one pH level, inhibition at another, depending on the concentration of the undissociated form. It is shown in Table I that this is the case.

TABLE I.

Effect of change in pH on action of a given dose of DNP on respiration of commercial yeast. Yeast suspended in glucose phosphate of stated pH.

pH	Concentration of DNP mg. %	Concentration of undissociated DNP millimols X10^{-3}	Oxygen consumption cmm. per 10^8 yeast cells per hour
6.8	0	0.	173.2
6.8	40	2.96	242.5
6.0	0	0.	155.1
6.0	40	18.5	59.3

Finally, we have determined the effect of change in pH of the medium during a run on the physiological action of DNP. It is shown in Fig. 1 that a given total concentration which causes stimulation at pH 6.8 evokes marked inhibition when enough acid is added from the sidearm of the Warburg vessel to reduce the pH to 5.4. Here the same cells and the same concentration of DNP are

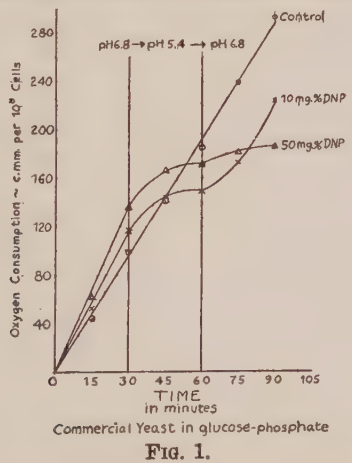


FIG. 1.

present throughout, but the increase in the concentration of the undissociated form consequent upon the decreased pH brings about prompt inhibition of respiration. Partial recovery occurs on restoration of the original pH level in the lower DNP concentration range. The control was not affected appreciably by the pH shift.

We have now shown on commercial yeast that a given total concentration of DNP will produce stimulation or inhibition of respiration depending upon the pH level, while the concentration of undissociated DNP is quite constant when optimal stimulation is evoked, and that change in acidity during a run will modify or reverse the effect of DNP on yeast respiration in a manner most directly explicable by changes in the concentration of undissociated DNP. We therefore conclude that in commercial yeast suspensions as in suspensions of pure yeast culture,[4] DNP stimulates or inhibits yeast respiration only in the undissociated form. We know of no evidence to the contrary.

7957 P

Production of Superovulation in Normal Immature Rats by Injection of the Principle in Menopause Urine.

HERBERT M. EVANS AND MIRIAM E. SIMPSON.

From the Institute of Experimental Biology, University of California.

The study of the effects of urine collected from a considerable number of normal women in the menopause and after castration has led us to regard the presence of lutein tissue in the ovaries of normal test rats as a common finding. The 24-hour specimens from such women were precipitated by alcohol, extracted with water and centrifuged. One-third of the powder thus obtained from each case was injected over a period of 3 days into a group of three 24 to 25 day old rats. (The remaining two-thirds was combined with synergist or pregnancy prolan in further tests for the active components of menopause urine, as will be seen in the following communication.) Autopsy was performed 96 hours after onset of injection. The urine of some of the patients was examined repeatedly; of the 20 women examined, the urine of 14 stimulated corpora on at least one occasion. Of a total of 88 tests, 20 showed corpus production.

We have, furthermore, been surprised to encounter ovarian weights of from 70 to 140 mg. in cases in which corpora lutea were

produced. There are many corpora in such ovaries—not the small crop characterizing pregnancy prolan. The histological picture of many of these corpora resembled that of the earliest stages of normal corpora just after shedding of the egg, and this fact together with the presence of distended oviducts led us to section the latter, where ova were encountered. In one case 83 eggs were found in 2 oviducts.

Smith and Engle[1] first reported experimentally induced superovulation in normal immature mice and rats after the implantation of rat anterior pituitary tissue. The phenomenon was again encountered by Leonard and Smith[2] on administering to hypophysectomized rats a mixture of the gonadotropic substances found in menopause and pregnancy urines respectively.

Irrespective of the interpretation or explanation adopted, menopause urine when administered alone is here shown to contain not infrequently a hormone or hormones capable of causing luteinization and, moreover, superovulation in normal immature test animals.

7958 P

Synergism or Augmentation Produced by the Addition of an Hypophyseal Synergist to Menopause or Castration Urine.

HERBERT M. EVANS AND MIRIAM E. SIMPSON.

From the Institute of Experimental Biology, University of California.

Corpora lutea are frequently produced in immature test animals by the administration of menopause or castration urine alone; indeed, superovulation may result. It is to be admitted that the effect of the majority of such urines is characterized by the growth of follicles only in the test animals. Nevertheless, most of the latter urines also produce abundant corpora and moderately large ovaries in test animals when combined with an hypophyseal fraction (synergist) which by itself gives infantile ovaries in the 96-hour period. This phenomenon has been observed in 30 out of 34 tests on 20 castrate or menopause patients. The ovaries stimulated by this combination weighed from 50 to 150 mg.; the synergism reached as much as 290% and averaged 108%.

1 Smith, P. E., and Engle, E. T., *Am. J. Anat.*, 1927, **40**, 159.

2 Leonard, S. L., and Smith, P. E., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 283.

7959 P

A Sensitive Biological Test for Menopause or Castration Prolan.

HERBERT M. EVANS AND MIRIAM E. SIMPSON.

From the Institute of Experimental Biology, University of California.

Low amounts of menopause or castration prolan which are difficult to recognize by the usual tests can easily be recognized by combining this substance with definite amounts of pregnancy prolan. The level of pregnancy prolan chosen is a low one but is always somewhat in excess of the minimal effective level when administered by itself. By the employment of this synergic reaction, rats prove to be just as sensitive test objects as are mice for the recognition of menopause or castration prolan.

A single example may be given. The administration of a gram of the alcohol precipitate from the urine of a castrated woman injected into 3 immature female rats, gave ovarian weights averaging 26 mg. and small or medium follicles only. When combined with levels of pregnancy prolan which alone gave ovarian weights averaging 30 mg., the combination yielded ovarian weights averaging 150 mg. In this case the hormone could not have been recognized in rats without the synergic reaction. It is remarkable that the "synergic" ovaries consist predominantly of follicles.

7960 P

Gonadotropic Effects in Hypophysectomized Female Rats of Implants of Pituitaries from Castrated Males.

HERBERT M. EVANS, MIRIAM E. SIMPSON AND RICHARD I. PENCHARZ.

From the Institute of Experimental Biology, University of California.

Studies of female rats united parabiotically with castrated males have shown that they are characterized by constant estrus and that their ovaries contain only large follicles (Witschi and others). The phenomenon appears to establish the actual secretion into the blood stream of the follicle stimulating hormone only on the part of the pituitary of the castrated male parabiont. The present study shows that the pituitaries of such castrated males nevertheless contain or "house" appreciable amounts of luteinizing hormone.

Young mature males were castrated and after 40 days their pituitaries were implanted into hypophysectomized females 26 days of age. While a dose level was found in which follicles only occurred in the ovaries of the hypophysectomized recipients, double this dose (4 glands) led to the appearance of corpora lutea. Implants of the hypophyses from normal litter brothers produced only follicles at both dose levels. Parallel experiments with normal recipients showed that corpora were produced by both levels of castrate and normal hypophyses.

The luteinizing effect of castrate male hypophysis as tested by implantation, therefore, contrasts with the results obtained by parabiosis. The explanation offered for the difference is that the absorption of the implant frees the factor responsible for luteinization, whereas this substance is retained *in vivo* by the hypophysis of the parabiont.

7961 P

Detection of Mammotropin* in the Urine of Lactating Women.

WILLIAM R. LYONS AND EMERY PAGE. (Introduced by Herbert M. Evans.)

From the Division of Anatomy, University of California.

In the course of studies on the hormonal control of the mammary gland, it has been of interest to ascertain by urinalysis to what extent a lactating woman is under the influence of estrin and the hypophyseal mammotropic hormone. A crude estrin may be prepared from the urine and tested by smearing it in the vaginae of ovariectomized rats. The urines of 8 lactating women (4-13 days postpartum) have been tested and all found to contain mammotropin in amounts that make it appear that at least as much of this hormone is excreted daily as is extractable from a bovine anterior lobe. The urine may be treated as follows: (1) to 100 cc. add 200 cc. acetone and 3 cc. HCl (concentrated); centrifuge and discard insoluble material; (2) add acetone to 90%; discard supernatant; (3) extract precipitate with mixture of 10 cc. stronger ammonia water, 20 cc. water, and 60 cc. acetone; discard insoluble; (4) add one volume of acetone; discard supernatant; (5) wash precipitate with 85% acetone, absolute acetone and ethyl ether (2 x 25 cc. in each

* Lactogenic hormone, prolactin, galactin.

case); (6) dry precipitate in warm desiccator; dissolve in 5.0 cc. water; adjust to pH 7.6; discard any insoluble.† Inject in doses of 0.1 and 1.0 cc. intradermally over the right and left crop sacs of squabs one month from hatching‡ for presumptive testing. Sacrifice birds at 48 hours, and retest in accordance with results until the minimal effective dose is determined. Until the hormone is purified it may suffice to consider the minimal effective dose in a statistically adequate number of birds as the temporary unit of urinary mammotropin. Sufficient amounts of untreated urine or blood plasma may be injected into the crop areas to allow for detection of mammotropin, but this procedure sometimes prevents accurate reading of the reactions because of inflammatory processes.

7962 P

Is Thyrotropic Hormone of Beef Ant. Pituitaries Identical with Indirect Interrenotropic Factor?

MORVYTH MCQUEEN-WILLIAMS. (Introduced by Herbert M. Evans.)

From the Anatomical Laboratory, University of California.

The ratio of the amounts of thyrotropic hormone in rat and beef hypophyses is entirely different from the ratio of the interrenotropic content in pituitaries of these 2 animal forms. Per unit of weight, adult male rat hypophyses are 7 to 9 times as potent in thyrotropic hormone as beef glands, whereas bovine pituitaries exceed male rat hypophyses in the ability to hypertrophy the adrenal cortex of adult male rats.

Intramuscular implants into immature male guinea pigs and subsequent histological study of the thyroids showed that as good a response can be elicited with 6 mg. of male rat pituitary as with 50 mg. of beef.

The adrenal weight is almost doubled when 1800 mg. of bovine glands are implanted over a 5 to 10 day period into adult male rats,

† If large volumes of urine are to be worked up, evaporate to dryness over a steam bath and under negative pressure, remove salts, and substances soluble in absolute fat solvents and proceed as above.

‡ Our method of local administration of mammotropin allows for its detection in a single microgram dose, whereas, when given intrapectorally (Riddle, *et al.*, PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1211) 100 microgram amounts are required.

provided the recipients have not been thyroidectomized.[1] On the other hand, as many as 200 (about 1600 mg.) adult male rat hypophyses implanted into intact rats produced no adrenal enlargement whatsoever; however, 350 whole glands administered to one rat did induce adrenal hypertrophy. Preliminary work indicates that adrenalectomy greatly increases the interrenotropic capacity of rat pituitaries, which, as we have just seen, is normally very low compared with the thyrotropic content.

7963 C

Sex Comparison of Gonadotropic Content of Anterior Hypophyses from Rats Before and After Puberty.

MORVYTH MC QUEEN-WILLIAMS. (Introduced by Herbert M. Evans.)

From the Anatomical Laboratory, University of California.

One hundred and eleven immature female rats (Table I) were each implanted intramuscularly with 2 to 10 (usually 7) anterior pituitaries from male or female rats of 5 main age groups. In parentheses will be given the average ovarian weight induced by each group when implants of 7 hypophyses were made.

18-23 *days:* Female rat pituitaries are very potent (73 mg.), while the level of gonadotropic hormone is much lower in male hypophyses (27 mg.).

27-30 *days:* Female glands still show a high hormonal content (88 mg.). A prepubertal rise in gonad-stimulating capacity has taken place in the male pituitaries (73 mg.).

35-38 *days:* A sudden prepubertal drop in the amount of hormone has occurred in both male (29 mg.) and female (32 mg.) hypophyses. Note that although the pituitaries are heavier, they have lost two-thirds of their potency in the period of one week.

42-44 *days:* Male remains the same, but female pituitary potency has decreased. More than half the female rats in this group had just matured.

Over 4 *months:* Adult female hypophyses cause but slight ovarian enlargement (25 mg.) and rarely corpora in the immature recipients, whereas male pituitaries induce ovaries 3½ times as heavy (84 mg.), which is in accord with the work of Evans and Simpson.

[1] McQueen-Williams, M., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 296.

TABLE I.
Age and Sex Differences in Gonadotropic Potency of Rat (Long-Evans) Pituitaries.

	Donors (never injected or mated)		Rat Pituitaries implanted into each recipient		Recipients (24-day ♀ rats)	
Age (days)	Sex	Aver. wt. 2 Gonads (mg.)	Total wt. (mg.)	No.	Aver. wt. 2 Ovaries (mg.)	No.
18-21	♀	9.9	18.5	7	74	7
22-23	♀	15.3	19.5	7	71	4
27-28	♀	16.1	22.2	7	90	7
27-29	♀	—	23.0	9	83	1
28	♀	—	16.0	6	73	1
28-29	♀	18.6	22.8	7	84	4
33-35	♀	—	18.0	6	21	1
36-37	♀	21.5	30.5	7	32	4
42-44	♀	29.7	36.4	7	25	7
4-10 mos.	♀	58.4	80.1	7	25	4
7 "	♀	—	—	6	27	12
7 "	♀	—	—	5	21	3
21-23	♂	221.0	17.1	7	27	6
23	♂	—	—	5	23	4
28-30	♂	504.0	23.1	7	73	6
31	♂	—	—	8	90	3
35-38	♂	627.4	32.7	7	29	6
44	♂	—	—	5	23	3
44	♂	—	—	10	54	1
4-6 mos.	♂	3000.0	64.8	7	84	7
7 "	♂	—	—	2	33	20

Swezy[1] has already mentioned my finding of the prepubertal fall, which led me to a series of further experiments on hypophysectomized rats. She also reported that, according to my experiments, hypophyses from male rats 1-13 days old produced no appreciable effect on the gonads or seminal vesicles of hypophysectomized male rats, but that by day 21 the male pituitary had attained a high potency, somewhat greater than that of the adult male hypophysis, per unit of weight, when tested on hypophysectomized rats. The present communication indicates that at 28-31 days the male pituitary reaches its *highest potency,* at least when tested on immature female rats. 18-28 day female hypophyses are equal to the male at its highest. After the prepubertal drop, the female pituitary steadily declines in its capacity to induce large ovaries in immature recipients, but the male later regains most of its former potency.

[1] Swezy, O., *Endocrinol.*, 1934, **18**, 619.

7964 C

Effect of a Low Calorie, Low Protein Diet on Blood Proteins.

WINDSOR C. CUTTING AND RICHARD D. CUTTER. (Introduced by Arthur L. Bloomfield.)

From the Department of Medicine, Stanford University School of Medicine.

The function of the labile protein of the blood is almost as poorly understood in the rat where it has to some extent been studied as in man where its workings are largely a matter of conjecture. Bloomfield,[1] and Torbert[2] have shown that starved rats lose a small percentage of plasma protein in from 4 to 6 days, and then stay constantly at the reduced level until death. This easily lost protein, largely albumin, is the labile fraction.

To study the same problem in man 26 normal subjects were placed for 2 days on a diet containing only 500 calories per day, and no protein. Fluids were not restricted. The protein content of the blood serum, taken in the fasting state, was determined before and after the experimental period by the method of Barnett, Jones and Cohn.[3]

Surprisingly enough, instead of falling, the total serum protein concentration rose during the test. The average value for the 26 subjects at the start was 7.40 gm. of protein per 100 cc. of serum. At the end it was 7.74 gm. per 100 cc. The individual values are shown in Table I.

To see whether or not the increased protein concentration was due to concentration of the blood, blood volume estimations were made on 9 of the subjects before and after the test diet, using the method of Keith, Rowntree and Geraghty.[4] The plasma volume fell consistently, the average at the start being 2,366 cc., and at the end being 2,058 cc., showing that the blood was being concentrated. However, the total protein of the plasma, as obtained from the product of the concentration and the volume, fell slightly, the average before being 170.7 gm., and afterwards 156.5 gm. This indicated that in spite of the increased protein concentration a small amount of protein was being lost.

An average of 4 pounds of body weight was lost during the 2 days of the test. This was apparently due to the combination of

1 Bloomfield, Arthur L., unpublished data.

2 Torbert, Harold C., in press.

3 Barnett, C. W., Jones, R. B., and Cohn, R. B., *J. Exp. Med.*, 1932, **55**, 683.

4 Keith, Rowntree and Geraghty, *Arch. Int. Med.*, 1915, **16**, 547.

TABLE I.
Protein Concentration and Blood Volume before and after 2 days of protein starvation.

Start 7.68 g/100cc.	Finish 7.42 g/100cc.	Plasma Volume Start	Plasma Volume Finish	Total Plasma Protein Start	Total Plasma Protein Finish
8.23	8.29				
7.99	7.94				
6.88	7.27				
7.18	7.60				
8.03	8.45				
6.82	7.38				
7.11	7.81				
7.57	7.55				
7.14	7.40				
7.74	8.03				
7.39	8.10				
7.96	8.44				
7.43	8.39				
8.15	8.10				
7.53	7.71				
6.28	6.53				
7.25	8.11	2400 cc.	2055 cc.	174.0 gm.	166.6 gm.
7.02	7.60	4180	3340	293.3	253.6
7.43	8.02	2370	2277	176.2	182.5
7.00	7.15	2995	2220	210.0	158.0
6.51	6.90	2145	1978	139.8	136.5
7.15	7.42	2220	2180	158.0	161.5
8.40	8.13	1900	1605	159.5	130.5
7.74	8.41	1544	1353	119.4	113.8
6.90	7.00	1542	1511	106.3	105.7
Av. 7.40	7.74	2366	2058	170.7	156.5

lack of food intake, loss of water, and perhaps some tissue destruction. The subjects were as a rule hungry throughout the experiment, at times to the point of nausea, and they drank little water unless forced.

It was apparent, therefore, that 2 days of protein starvation in man concentrated the blood as evidenced by increased protein concentration and lessened volume, and that it slightly lowered the total protein content.

In order to investigate the factors which caused the blood concentration several further procedures were carried out.

That activity was not involved was shown by the fact that of the 26 subjects 10 were in bed for several days before and during the test; 8 were very active in their duties as house officers throughout the test; and 8 changed from some sort of activity to bed rest for the experiment. The rise was practically uniform in all these groups.

Since it is the blood proteins of small molecular size that exert the highest osmotic pressure, a decrease of these proteins greatly reduces the ability of the blood to hold water. It seemed that a small amount of this fraction might have been used, and so have

reduced the oncotic pressure of the serum out of proportion to its weight, with resultant concentration. The oncotic pressures were measured in 3 subjects by Dr. Garnett Cheney who found that the serum oncotic pressure did not change significantly during the 2 days of the test. The average oncotic pressure at the start of the diet was 300 mm. of water, and at the end was 296 mm. of water. A slight increase of oncotic pressure would have been expected with the more concentrated plasma unless some protein were lost, and so this may be further evidence of protein loss.

However, it could not be shown by fractionating the proteins that there had been any changes in the relative concentrations of any of the proteins. Dr. Eloise Jameson separated the pooled sera from 7 subjects into the various protein fractions. The concentration curves including the smallest molecule fractions were identical before and after the experiment. Individual protein fraction loss, therefore, as a mechanism for lowering osmotic pressure and thus concentrating the blood seemed to be of no importance.

The test diet contained only one gram of salt per day, but salt deprivation was not responsible for the blood concentration, since when 7.5 gm. of salt per day were added to the diet, the usual fall of blood volume was noted in 2 subjects. The average plasma volume before the experiment was 2,112 cc., and afterwards 1,931 cc. In 2 other subjects who were given diets of 2,400 calories, but salt free, the fall in volume did not occur. The average before was 2,413 cc. and afterwards 2,405 cc.

It was presumed that the low food, and particularly the low fluid, intake might be important. In 2 subjects, therefore, fluids were forced to 3,000 cc. and the changes in blood volume found to be diminished. The average volume before the test was 2,205 cc. and after it 2,155 cc. Dehydration is therefore an important factor in the blood concentration.

That the blood should become concentrated during a time when the diet approaches starvation, unless large amounts of fluids are taken, is credible. Why the total amount of protein should decrease and yet retain its previous character absolutely, however, is less apparent, especially since it is well known that patients with edema due to low plasma protein have a reduction in albumin out of proportion to that of globulin. It must be presumed that this change of total plasma protein indicates a diminution of all tissue proteins to a level commensurate with the true decrease in body weight. This is analogous to the natural changes of growth, or those of a wasting disease in which body weight, and therefore its corollary, blood

volume, changes and the total protein changes correspondingly. The 2 days of protein deficiency apparently represent these slow and more or less natural changes in an accelerated form and are insufficient to cause alteration in the relationships of the blood protein fractions, which longer starvation would probably expose. It would seem that the loss of body weight is probably due in part to the tissue destruction which reduces total serum protein, but principally to dehydration, since a high fluid intake minimizes it.

Conclusions. 1. Due principally to low fluid intake, the blood is concentrated and the serum protein concentration slightly less than proportionately increased by a low calorie diet containing no protein.

2. Two days of protein deprivation is an insufficient time to make any change in the character of the blood proteins, but lowers the total blood protein definitely.

7965 C

Stimulation of Adrenal Medulla by Irradiated Insulin.*

J. MURRAY LUCK AND GORDON M. RICHMOND. (With the assistance of J. von Saltza.)

From the Biochemical Laboratory, Stanford University.

Davis, Luck, and Miller[1] showed that insulin on long exposure to soft X-rays of high intensity loses its characteristic ability to lower the blood-sugar content and, in massive doses, may even produce a slight degree of hyperglycemia. A substantial portion of its phosphate-lowering activity is retained, as well as part of its amino-acid-lowering activity. It was suggested, by way of explanation, that the irradiated insulin, though otherwise inactivated, retains its power to stimulate the adrenal medulla, thus causing the slight hyperglycemia, hypophosphatemia, and hypoaminoacidemia actually observed.

To test this hypothesis we have administered insulin, 7 to 10 units per kilo, irradiated with soft X-rays for 3 hours at an intensity of 3,400 Roentgen units per second, to adreno-demedullated rabbits. Six animals were employed, and in no case was a lowering in blood

* We are greatly indebted to Professor H. Jensen for the crystalline insulin and to Mr. Morden G. Brown for operating the X-ray equipment. The adreno-demedullated rabbits were generously provided by Professor J. E. Markee.

[1] Davis, B. L., Jr., Luck, J. M., and Miller, A. G., *Biochem. J.*, 1933, **27**, 1643.

amino acids observed. In 5 normal animals the irradiated insulin caused quite a pronounced decrease (Table I). Quantitatively the

TABLE I.
Effects of irradiated insulin on demedullated rabbits.

NORMAL					ADRENO-DEMEDULLATED				
Post-injection values after 1.5 and 3 hr.					Post-injection values after 1.5 and 3 hr.				
		% initial					% initial		
Amino N		Blood Sugar		Insulin	Amino N		Blood Sugar		Insulin
1.5	3	1.5	3	used	1.5	3	1.5	3	used
97	89	79	110	Cryst.	99	99	100	101	U- 80
86	75	61	92	”	97	101	96	100	U-100
92	83	69	81	”	101	100	101	101	U-100
87	89	88	98	”	99	100	89	96	Cryst.
78	86	81	98	”	99	99	92	98	”
					98	99	87	93	”

results differ from those obtained by Davis, Luck, and Miller in that no tendency towards hyperglycemia was observed. Indeed, for reasons that are still obscure, we were unable to *totally* destroy the hypoglycemic activity of the insulin, despite substantial increases in irradiation time and intensity. In the adreno-demedullated rabbits the irradiation product was completely inactive, such hypoglycemia as was observed being confined to the normal animals.

The results clearly indicate that the irradiated insulin, though inactive to a large extent wth respect to blood sugar, retains a substantial portion of its activity with respect to blood amino acids. The facts accord with the hypothesis that the irradiation of insulin by soft X-rays does not destroy its ability to stimulate the adrenal medulla.

7966 C

A Comparison of the Resistance of Bacteria and Embryonic Tissue to Germicidal Substances. III. Mercurochrome.

A. J. SALLE AND A. S. LAZARUS.

From the Department of Bacteriology, University of California, Berkeley.

Lambert and Meyer[1] bathed fragments of rabbit spleen in a suspension of *Staphylococcus aureus* for one minute followed by immersion in graded solutions of mercurochrome for 20 minutes.

1 Lambert, R. A., and Meyer, J. R., PROC. SOC. EXP. BIOL. AND MED., 1926, **23**, 429.

Tissue cultures were then prepared in hanging drops of homologous plasma, following 2 washings of the tissue in physiological salt solution. A second set of hanging drop cultures was made from non-infected tissues similarly exposed with appropriate controls of untreated tissues. Mercurochrome in a dilution of 1-250 killed *Staphylococcus aureus,* 1-500 killed fragments of spleen tissue.

German[2] bathed skin of chick embryos in solutions of mercurochrome for one and 5 minutes. They were washed in Locke's solution, then embedded in a mixture of plasma and embryonic extract. For the bacterial tests, fragments of muscle and fascia were bathed in a broth culture of *Staphylococcus aureus* followed by immersion in the various dilutions of mercurochrome. After periods of one and 5 minutes the fragments were planted on agar plates. "Efficiency indexes" were determined by multiplying the percent of tissue cultures showing growth by the percent of bacterial cultures showing inhibition at the same concentration. Mercurochrome gave an efficiency index of 0.0132 (perfect germicide = 1.00).

Buchsbaum and Bloom[3] prepared chick tissue cultures in which the various dilutions of mercurochrome were embedded in chick plasma. The test organism, *Staphylococcus aureus,* was added to the embryonic fluid. The cultures were observed for bacterial and tissue growth after 24 and 48 hours' incubation. They stated that an antiseptic killing the bacteria at concentrations that would not harm the cells would have an index of 1.0 or greater (greatest dilution that killed the organisms divided by the greatest concentration in which cells show approximately normal growth). Mercurochrome was given an index of 0.5.

In previous papers in this series[4, 5] comparisons were made of the resistance of *Staphylococcus aureus* and embryonic chick heart tissue to Merthiolate, Metaphen and phenol. Toxicity indices were determined by dividing the highest dilution of germicide showing no growth of the embryonic chick heart fragments in 48 hours by the highest dilution capable of killing *Staphylococcus aureus* in 10 minutes but not in 5. Toxicity indices were as follows: Metaphen 12.7; phenol 12.9, and Merthiolate 35.3. Metaphen showed a *Staphylococcus aureus* phenol coefficient of 92; Merthiolate gave a coefficient

[2] German, W. J., *Arch. Surg.*, 1929, **18**, 1920.

[3] Buchsbaum, R., and Bloom, W., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 1060.

[4] Salle, A. J., and Lazarus, A. S., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 665.

[5] Salle, A. J., and Lazarus, A. S., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 937.

of 71. Theoretically, the smaller the toxicity index the more nearly perfect the germicide.

The methods followed were the same as those given in the first paper.[4] A *Staphylococcus aureus* phenol coefficient was first determined by the method of Reddish, followed by the addition of graded amounts of mercurochrome to embryonic chick heart tissue embedded in plasma and contained in Carrel flasks.

The highest dilution of phenol required to kill *Staphylococcus aureus* in 10 minutes but not in 5 was 1-65. For mercurochrome it was 1-40. This gave mercurochrome a *Staphylococcus aureus* phenol coefficient of 0.6.

Young, White and Swartz[6] found that mercurochrome in a concentration of 1-1,000 in urine killed *Staphylococcus aureus* in 1 minute; a 1-5,000 concentration in 5 minutes. Lancaster, Burnett and Gaus[7] reported that a 1-1,000 concentration of the germicide in Ringer's solution showed no growth of *Staphylococcus aureus* in 6-10 minutes. In the presence of serum, however, the activity was greatly decreased, a 1-100 solution required one hour to kill the organism. Scott and Hill[8] stated that a 1-60 dilution of mercurochrome in alcohol-acetone-water killed *Staphylococcus aureus* in one minute. Birkhaug[9] reported a *Staphylococcus aureus* phenol coefficient of 1.7. On the other hand Simmons[10, 11] stated that a 1-50 dilution of the germicide failed to kill the above organism in 10 minutes.

The tissue culture results are summarized in Table I.

TABLE I.

Germicide	Highest Dilution Showing no Tissue Growth = A	Highest Dilution Showing no Growth of *Staphylococcus aureus* = B	Toxicity Index = A/B	*Staphylococcus aureus* Phenol coefficient
Phenol	1-840	1-65	12.9	
Mercurochrome	1-10,500	1-40	262.0	0.6

It is seen that mercurochrome possesses considerable toxicity when tested by the tissue culture method. The germicides so far

[6] Young, H. H., White, E. C., and Swartz, E. O., *J. Am. Med. Assn.*, 1919, **73**, 1483.

[7] Lancaster, W. B., Burnett, F. L., and Gaus, L. H., *J. Am. Med. Assn.*, 1920, **75**, 721.

[8] Scott, W. W., and Hill, J. H., *J. Urol.*, 1925, **14**, 135.

[9] Birkhaug, K. E., *J. Am. Med. Assn.*, 1930, **95**, 917.

[10] Simmons, J. S., *J. Inf. Dis.*, 1926, **39**, 273.

[11] Simmons, J. S., *J. Am. Med. Assn.*, 1928, **91**, 704.

studied may be placed in the following order on the basis of their toxicity indices: Metaphen 12.7; phenol 12.9; Merthiolate 35.3; and mercurochrome 262.0.

Scott and Hill[8] stated that a 1-50 dilution of mercurochrome in alcohol-acetone-water "has a relatively low toxicity as shown by the vigorous way that tissue cultures and transplants have grown after its use." von Oettingen, Calhoun, Badertscher and Pickett[12] reported that the tissue toxicity of mercurochrome was relatively low, but a 5% aqueous solution was distinctly injurious as judged by excised ciliated mucous membranes.

On the basis of the above results it is concluded that mercurochrome is relatively toxic and rated considerably poorer than any of the germicides so far studied when tested by the tissue culture technique.

7967 P

Effect of 1-2-4 Dinitrophenol on Oxygen Uptake of Rat Tissue.

EDWARD MUNTWYLER.

From the Department of Biochemistry, School of Medicine, Western Reserve University, Cleveland.

Since Cutting and Tainter[1] and Magne, Mayer and Plantefol[2] have observed that small quantities of dinitrophenol cause a marked acceleration of the metabolism of animals, it seemed desirable to determine whether this drug accelerates the oxygen uptake of excised tissue.

The experiments here reported are a continuation of those given in a previous preliminary report.[3] The preparation of the rat tissue slices and the measurement of the oxygen uptake was done as previously described,[4] the tissue being suspended in glucose phosphate (pH 7.4) Ringer's solution. Four Warburg vessels were employed in each instance, 2 serving for the control observations. The fol-

[12] von Oettingen, W. F., Calhoun, O. V., Badertscher, V. A., and Pickett, R. E., *J. Am. Med. Assn.*, 1932, **99**, 127.

[1] Cutting, W. C., and Tainter, M. L., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1268.

[2] Magne, H., Mayer, A., and Plantefol, L., *Ann. Physiol. Physicochem. Biol.*, 1932, **8**, 1.

[3] Muntwyler, E., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 621.

[4] Muntwyler, E., and Binns, D., *Am. J. Physiol.*, 1934, **108**, 80.

lowing experiments were performed. The oxygen uptake of rat liver, kidney and muscle tissue obtained from rats previously injected with dinitrophenol was determined when the tissue was suspended in normal Ringer's solution. Three or more animals were employed in each case. The oxygen uptake of rat liver and kidney tissue obtained from normal animals was also determined when suspended in Ringer's solution containing the drug. In these experiments 2 or more animals were employed for each concentration of the drug. The oxygen uptake of normal rat liver was determined in 5 separate experiments when suspended in normal dog serum and serum from dogs previously injected with dinitrophenol. The response of the rat liver and kidney tissue in the presence of the drug was likewise compared with that obtained employing frog liver and kidney tissue.

The oxygen uptake of rat liver, kidney and muscle obtained from rats previously injected with dinitrophenol did not show an increased oxygen uptake when compared with tissue obtained from control uninjected rats. As a matter of fact, if anything, a slightly decreased rate of oxygen uptake was noted when the tissue from the treated animals was employed. Normal rat liver when suspended in Ringer's solution containing dinitrophenol (dinitrophenol, sodium salt was employed in the following concentrations: 0.005, 0.01, 0.05, 0.1, 0.5 and 1.0 mg. per 100 cc.) showed a questionable increase in the rate of the oxygen uptake in concentrations of the drug of 0.01, 0.05 and 0.1 mg. per 100 cc. (average maximum of 15% obtained with 0.01 mg. per 100 cc.). The oxygen uptake of rat kidney in the same concentrations of dinitrophenol showed no definite difference from the control. The oxygen uptake of rat liver suspended in serum obtained from dogs previously injected with the drug was not different from that of rat liver in normal dog serum. In confirmation of the work of Ehrenfest and Ronzoni[5] and De Meio and Barron[6] it was found that frog liver and kidney suspended in frog's Ringer's solution containing 0.5 mg. of dinitrophenol per 100 cc. showed a definitely increased rate of oxygen uptake. It should be pointed out that McCord[7] observed that dinitrophenol in a concentration of about 1-20,000,000 increases the oxygen uptake of rat liver and kidney (average increase of 18.4%

[5] Ehrenfest, E., and Ronzoni, E., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 318.

[6] De Meio, R. H., and Barron, E. S. G., PROC. SOC EXP. BIOL. AND MED., 1934, **32**, 36.

[7] McCord, W. M., *Am. J. Physiol.*, 1934, **109**, 232.

for liver and 20.3% for kidney), but decreases it in a concentration higher than 1-5,000,000.

7968 P

Blood Sugar Changes After 1-2-4 Dinitrophenol.

W. F. ASHE, JR. (Introduced by Edward Muntwyler).

From the Department of Biochemistry, School of Medicine, Western Reserve University, Cleveland.

It has been shown by a number of workers that 1-2-4 dinitrophenol induces a moderate but persistent hyperglycemia in animals.[1, 2, 3] A marked depletion of liver and muscle glycogen has also been reported.[1] The present experiments were done with the hope of obtaining further information on the effect of dinitrophenol on the blood sugar level.

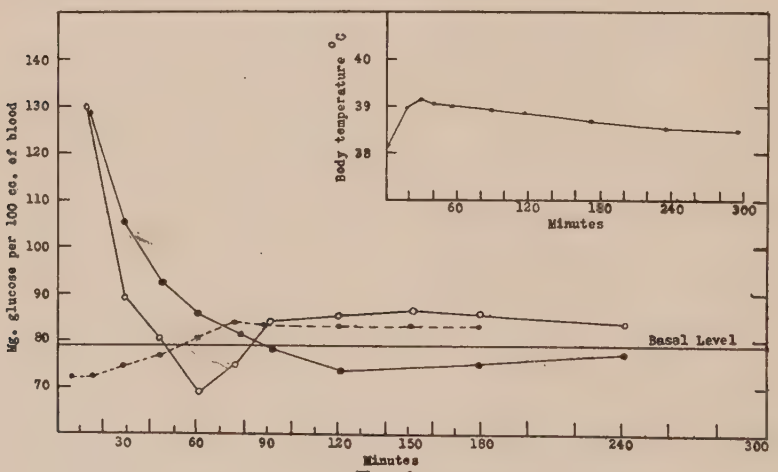


FIG. 1.

Rate of Glucose Removal from the Blood Following Intravenous Injection. o—o Rate of glucose removal from the blood when glucose was injected 30 minutes following DNP injection. ●—● Control rate of glucose removal from the blood. ●- - - ● Change in blood sugar following DNP injection (fasting condition).

1 Hall, V. E., Field, J., 2nd, Sahyun, M., Cutting, W. C., and Tainter, M. L., *Am. J. Physiol.*, 1933, **106**, 432.

2 Magne, H. Mayer A. and Plantefol, L., *Ann. Physiol. Physicochem. Biol.*, 1932, **8**, 1.

3 Hall, V. E., Brown, C. A., and Sahyun, M., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 380.

Two dogs were given subcutaneously 10 mg. of dinitrophenol (10 mg. sodium salt per kilo body weight were employed in all the subsequent experiments) per kilo body weight and the blood sugar level determined at various periods following the injection. It was found that approximately 30 minutes following the administration of the drug, the blood sugar began to rise (Fig. 1). Some 90 minutes after injection this rise ceased, and a new level was maintained for several hours.

In order to determine whether the injection of the drug altered the rate of removal of glucose from the blood, each animal was given intravenously 0.25 gm. of glucose per kilo body weight. The glucose was given as a 50% solution 30 minutes following subcutaneous injection of the drug and the blood sugar determined at 15 minute intervals. Figure 1 presents composite curves showing the rate of removal of the glucose from the blood with and without the previous administration of the drug. It will be noted that the rate of removal of glucose is more rapid when dinitrophenol had been given previously. It was observed that while this amount of glucose produced no glycosuria in the control, a small amount of reducing substances appeared in the urine in the first and second hours after dinitrophenol. The amount of reducing substance, if glucose, was insufficient to be entirely responsible for the more rapid disappearance of glucose from the blood after the dinitrophenol injection.

Similar injections of glucose were made 90 minutes following the subcutaneous injection of the drug. This time was selected since, as stated above, it was observed that about 90 minutes following the injection of the drug alone the blood sugar had ceased to rise and become constant. It was found that, although the initial rate of decline of the curve was comparable to that in the previous experiment, when the concentration of blood sugar fell to the level which would have been produced by the drug alone, it leveled out immediately and remained constant for several hours.

Summary. 1-2-4 dinitrophenol causes an hyperglycemia in dogs when injected subcutaneously. The drug also increases the rate at which excess glucose is removed from the blood after quantities of the latter have been given intravenously.

7969 P

Effect of Male Hormone on Protein Metabolism of Castrate Dogs.

CHARLES D. KOCHAKIAN. (Introduced by John R. Murlin).

From the Department of Vital Economics, University of Rochester.

Two castrated male adult dogs—No. 1 thin and very active, and No. 2 fat and inactive—were brought into weight and nitrogen equilibrium on a diet of beef heart, cracker meal, lard, cod liver oil, Wesson's salt mixture, and bone ash. The nitrogen intake of dog No. 1 was approximately 5.60 gm. per day, and of dog No. 2, 7.60 gm. per day. The amount varied somewhat with the nitrogen content of each beef heart, but was always determined by analysis. Periods of 5 days each were used.

The male hormone was prepared according to the Funk and Harrow method[1] and dissolved in olive oil so that 1 cc. of the oil solution was equivalent to 4 liters of urine or 40 bird units (B.U.). The hormone was administered subcutaneously twice per day, in the a.m. and p.m.

The results of 6 experiments are given in Table I.

TABLE I.
Summary of Nitrogen Balances. Gm. per period (5 days).

		Dog No. 1				Dog No. 2	
B.U. male hormone per day		20	40	60	40	40	60
Periods before injection	1	—0.10	+0.30	0.05	+0.65	+4.75	+0.45
	2			0.80	+1.30		+1.10
Injection periods	1	+1.45	+2.65	+2.55	+2.40	+4.50	+2.90
	2	+3.05	+3.45	+2.35	+3.10	+6.35	+6.45
	3	+2.85	+5.05	+0.50	+4.25	+6.45	+6.55
	4			+2.45		+6.60	+6.55
	5			+3.65			
Periods after injection	1	+3.15	+3.00	+1.30	+4.20*	+4.05	+3.90
	2	0.00	+0.75	—1.00	+1.90	+1.00	—2.75
	3		+0.55*	+0.65	+2.10	—1.75†	—1.50
	4			+1.30	+2.50	—1.30	—1.45
	5				+1.20	+0.40‡	—0.65

* Four-day periods, but calculated for 5 days.
†Three days lost between this and the preceding period.
‡Five more periods following but not recorded.

The results indicate in every experiment a definite and marked retention which is attained in the first injection period and maintained through the injection periods and into the first or second

[1] Funk, C., Harrow, B., and Lejwa, A., *Am. J. Physiol.*, 1930, **92**, 440.

after period. Furthermore, the same effect is produced with the different doses. The latter effect is shown very strikingly by dog No. 2, but not so clearly by dog No. 1 with the exception of experiment No. 1, and possibly No. 3.

Although there is an excess output of nitrogen after cessation of injections, it is not at any time comparable with the amount retained. Also to be noted in this respect is the fact that in both dogs a minus balance is reached sooner after the 60 B.U. than in the other experiments. However, this may not prove to be especially significant.

In every experiment the retention is due to a decrease in urinary nitrogen; none in fecal nitrogen. Partition of urinary nitrogen indicated a change in urea paralleling that of the urinary nitrogen, ammonia and creatinine remained constant, creatine increased the second period after cessation of injections.

In every experiment the dogs' weights showed a gradual and definite increase which continued into the first period after injections and then gradually returned to basal. Dog No. 2 showed a much greater increase in weight than dog No. 1.

No excessive thirst or diuresis was noted.

Whether the retention of nitrogen is due to the male hormone, *per se,* or to an indirect effect, e. g., the anterior pituitary growth hormone, cannot at present be definitely stated.

7970 C

Further Observations on the Action of Adenosine on the Perfused Heart.

ALFRED M. WEDD.

From the Department of Physiology, The University of Rochester School of Medicine and Dentistry.

In an earlier study[3] of the action of adenosine on the perfused heart of the rabbit it was believed that this substance improved the beat of the heart, although records which were obtained by attaching a lever to the ventricle never showed an increase in height of contraction. There was no change in amplitude provided the rate was kept constant. When the normal rhythm was slowed by adenosine there was a decrease in height of contraction due, as Dale[1] has

3 Wedd, A. M., *J. Pharmacol. and Exp. Therap.*, 1931, **41**, 355.

1 Dale, A., *J. Physiol.*, 1930, **70**, 455.

shown, solely to the slowing. Drury[2] later reported that on rare occasions adenosine caused a slight increase in the mechanogram, which usually occurred when the heart was in poor condition, and the coronary outflow was considerably increased by the injection. Wedd and Fenn[4] found that when adenosine was added to a bath in which was suspended an auricle or a strip of heart muscle, either cold-blooded or mammalian, depressed contractility invariably resulted. At times when injected into the rhythmically stimulated perfused heart adenosine has been seen to depress excitability and cause a short period of 2:1 response (Fig. 1). From observations

FIG. 1.

Perfused cat heart. Upper line, time, 5 seconds; middle line, Condon tipping bucket; lower line, myogram. 0.4 mg. adenosine injected at arrow.

of the effect on the muscle itself, it has been concluded that if adenosine were to improve the beat of the heart it must do so by its vaso-dilator action.

A series of experiments has recently been undertaken to study the influence of certain choline derivatives on the heart. The technique was similar to that previously employed. The hearts of 35 rabbits and of 7 cats have been perfused. In each experiment from one to 3 injections of adenosine were made to test the reactivity of the coronary vessels. These injections were usually given toward the end of the experiment when the heart was no longer in good condition and the coronary outflow had fallen off. Under such circumstances, almost without exception, when adenosine caused an increase in coronary flow the height of the mechanogram showed a definite, and often a marked, increase (Fig. 1). The reason for the failure to observe any influence on the force of contraction in earlier experiment is now apparent. Only nucleic acid derivatives with a vaso-dilator action were then investigated. It was pointed out that with large doses or repeated smaller doses the coronary vessels be-

[2] Drury, A. N., *J. Physiol.*, 1932, **74**, 147.

[4] Wedd, A. M., and Fenn, W. O., *J. Pharmacol. and Exp. Therap.*, 1933, **47**, 365.

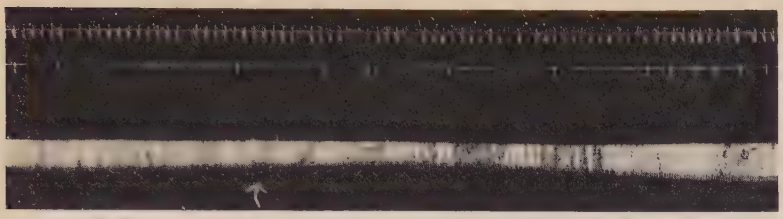


FIG. 2.

Perfused rabbit heart. Perfusion fluid contains acetyl-B-methyl-choline, concentration 1:50,000, which has slowed the flow and diminished the beat. 0.6 mg. adenosine injected at arrow.

came widely dilated and most preparations were abandoned because the vessels had become non-reactive in a state of maximum dilation. The rate at which the heart was maintained was just above the normal rhythm, approximately 120 to 150 beats per minute, and this together with a high or maximum coronary flow at all times, provided the most favorable conditions that could be established, and so the recorded beat was necessarily optimum as far as it could be affected by external factors. From more recent experiments it has become certain that when the condition of the heart is not good and the coronary flow has fallen off, the contraction may be expected to improve as a result of the increase in flow that follows adenosine. The value of adenosine in improving the condition of the perfused heart and prolonging the usefulness of the preparation has been repeatedly demonstrated in these experiments.

The power of adenosine to counteract the diminution in coronary flow produced by acetyl choline has been reported.[4] When the coronary flow and the size of the beat have been reduced by acetyl-B-methyl-choline, adenosine causes an increase in flow and an increase in the size of the beat (Fig. 2).

7971 C

Unusual Allergic Manifestations in B. Dysenteriae Infections.

DANIEL N. SILVERMAN AND BERNARD G. EFRON.

From the Department of Medicine, School of Medicine, Tulane University of Louisiana.

In a study of 125 cases of bacillary dysentery caused by various strains of *B. dysenteriae*, namely, Flexner, Duval, Shiga and Hiss-Y, it was noted that 4 of these presented symptoms that differed

from the usual case. These symptoms were those commonly noted in cases of bacterial protein hypersensitiveness. One of us (Silverman) suspected in these cases of chronic bacillary infection an allergic reaction, because of the peculiar manifestations following vaccine therapy. It was noted that shortly following the injection of small doses of the specific vaccine, the toxic symptoms of the acute infection were inaugurated, including an anaphylactic skin reaction at the point of the injection, and certain well defined constitutional allergic evidences. Of these, the most marked were those of the vasomotor mechanism. In 2 cases, the allergic reaction was so severe as to be alarming, in that the heart action became rapid and weak and there was a concurrent fall in the blood pressure. The temperature in these cases was always up following the injections but at no time was the elevation above 1° to 1½° above normal. Undoubtedly, the vasomotor disturbance together with the effect upon the heart muscle explained the near collapse which was noted for 2 of the cases. In one case, in which there was a return of the diarrhea, the dejecta contained considerable blood. This bloody mucus stool was identical with the typical stool of her acute bacillary colitis, which she had experienced 5 years previously. The question arose whether the return of blood in the dejecta meant a lighting up of the infection or the rupture of small vessels in the bowel as a result of allergy. Subsequent culture of these stools revealed the presence of *B. dysenteriae*. It is our opinion that the return of blood in the stool was due to vascular rupture (allergy) rather than to the return of infection. This belief was arrived at on the ground that a well marked cutaneous reaction occurred at the vaccine inoculation site, which reaction could only be allergic.

The allergic reaction seen in certain cases of chronic bacillary dysentery would seem to prove that the intestinal infection is still present, because in some cases of chronic bacillary dysentery, in which the specific organism was no longer detectable in the stool, there was no allergic skin reaction.

It is a well recognized fact that in all bacterial allergies the living antigen (micro-organism) is still in the host. Only in this way can the tissue hypersensitiveness be accounted for. For this reason, we believe that the cutaneous allergy in certain cases of bacillary dysentery is of considerable diagnostic importance. Certainly, it is a simple, sure method of diagnosis compared to that of the searching for the specific bacillus in the stool. Furthermore, it is far more reliable as a diagnostic point than the agglutination reaction because the latter is very often absent in chronic bacillary dysentery, and

when present is in such low dilutions as to be of little significance.

The allergic state seen for certain cases of chronic bacillary dysentery undoubtedly explains the failure of vaccine therapy in the cure of the infection. These cases can be advantageously treated with vaccine provided they are desensitized. Our experience substantiates this opinion, having after desensitizing the patients caused a disappearance of symptoms and the organisms from the stool following the administration of the vaccine.

The sensitivity is group specific. It follows closely the agglutination reaction, although it is not as individually specific as the agglutinins. The agglutination reaction is often positive only for the homologous organism; the skin test may be positive for different strains of the same organism. For instance, the Shiga organism isolated from Case 1, was agglutinated by the serum of the patient of Case 1, whereas skin reactions to this organism were obtained both in Case 1 and Case 2. Case 3 showed positive skin reactions to the same extent as the agglutination reactions. In both types of tests reactions were obtained to non-homologous strains.

TABLE I.

Case	Stool Organism Isolated	Agglutination
1	*B. dysenteria* (Shiga)	Positive against autogenous isolated organism, dilution 1/80
2	*B. dysenteria* (Shiga)	Positive, dilution 1/160, against autogenous isolated organism Shiga.
3	None of Dysentery Group	Positive for 1/40 and 1/80 dilution, *B. Flexner*, and for 1/40 Shiga.
4	*B. dysenteria* (Flexner)	Positive, dilution 1/160 against autogenous isolated organism.

Skin tests were made by the intracutaneous method, 1/100 cc. injected intradermally; readings were taken at 15 minutes and at 24 hours. Controls were used with each series of tests. A test was considered positive only when the wheal and erythema at the 15-minute reading was at least 2 to 3 times that produced by the con-

TABLE II.

Vaccine of Organism	Case No. 1	2	3	4
Staph. albus	—	—	—	—
B. Shiga (Case 1)	Pos.	Pos.	Pos.	—
B. Shiga (Case 2)	—	Pos.	Pos.	—
B. Flexner-Harris	—	—	Not used	—
B. Flexner (Stock)	—	—	Pos.	—
B. Duval	—	—	Not used	—
B. typhosus and Paratyph. A and B	—	—	—	—
B. Flexner (homologous)	—	—	—	Pos.

trols. The 24-hour reaction was recorded but was not used as a criterion of sensitivity in this study.

The vaccines as a rule contained around three billion organisms to the cc. Tests were made with serial dilutions, using a 1/10, 1/100, 1/1000 dilution of these vaccines.

TABLE III.
Agglutinations.

	Case 1			Case 2			Case 3			Case 4		
	1/40	1/80	1/160	1/40	1/80	1/160	1/40	1/80	1/160	1/40	1/80	1/160
B. Hiss (Stock)	—	—	—	—	—	—	Not done			—	—	—
B. Duval (Stock)	—	—	—	Not done			"	"		—	—	—
B. Flexner (Stock)	—	—	—	—	—	—	Pos.	Pos.	—	—	—	—
B. Shiga (Stock)	—	—	—	—	—	—	Pos.	—	—	—	—	—
B. Shiga (Case 1)	Pos.	Pos.	—	—	—	—	Not done			—	—	—
B. Shiga (Case 2)	—	—	—	Pos.	Pos.	Pos.	"	"		—	—	—
B. Flexner (Homologous)	—	—	—	—	—	—	—	—	—	Pos.	Pos.	Pos.

Summary. We believe we have demonstrated atopic (anaphylactic type) sensitivity for *B. dysenteriae,* occurring during course of infestation or infection of the bowel with these organisms. We know of no previous report. Cook's postulates have been fulfilled by: 1. Positive cutaneous reactions with the vaccines. 2. Reproduction of symptoms by subcutaneous injections of the vaccines. 3. The organism was isolated from the individual (in 3 cases; in the 4th serological evidence indicated the patient's contact with the organism).

7972 P

Sugar Utilization in Hypophysectomized Rabbits.

PAUL O. GREELEY. (Introduced by D. R. Drury.)

From the Department of Physiology, School of Medicine, University of Southern California.

The fact that serious hypoglycemia may develop in hypophysectomized rabbits and dogs is well known.[1-4] In preliminary experiments it was found that glucose must be administered to fasted

1 Corkill, A. B., Marks, H. P., White, W. E., *J. Phy.*, 1933, **80**, 193.

2 White, W. E., *Proc. Royal Soc.* (Series B), 1933, **114**, 64.

3 Mahoney, W., *Am. J. Phy.*, 1933, **109**, 3.

4 D'Amour, M. C., Keller, A. D., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1175.

hypophysectomized rabbits to prevent hypoglycemic convulsions and death. The animal requires large amounts of sugar even when the blood sugar level is low. The work reported here was concerned with the measurement of this requirement. The aim was to determine the minimum amount of glucose necessary to maintain a proper blood sugar level in fasted hypophysectomized rabbits. After a 3-day fast the pituitary was removed by an oral approach (Cope's[5]) modification of White's method.[2]

Frequent blood sugar estimations were carried out after the hypophysectomy. When the level had fallen to 80 mg. % or lower sufficient 10% glucose was given intravenously every hour thereafter to keep it from going lower. The rate of injection was adjusted according to whether the previous blood sugar had indicated the level was rising or falling.

The interval between time of operation and time when the blood sugar began to drop varied in different rabbits from 11 to 32 hours. The amount of glucose required increased during the first 5 to 8 hours thereafter, by which time a maximum requirement was attained. There was little increase above this. Glucose was given in some cases by continuous intravenous injection. In a given animal, the amount required was the same whether the glucose was given at one hour intervals or by continuous injection. Urinary determinations for sugar were uniformly negative. The amounts of glucose which had to be injected during the period of maximum need is recorded in grams per kilo body weight per hour in Table I.

TABLE I.

Wt. kilo	Hr. after hypophysectomy when blood sugar began to fall	Hr. after hypophysectomy when rate was determined	Glucose intravenously in gm. per kilo body wt. per hr.	Remarks
1.59	12	18	.503 6 hr.	continuous
2.27	18	27	.554 5 "	"
2.84	18	24	.601 6 "	"
3.30	24	29	.642 6 "	"
1.87	12	22	.671 6 "	"
1.96	16	32	.510 3 "	intermittent
1.70	21	23	.617 5.5 "	continuous
1.82	—	18	.527 5 "	intermittent

It is apparent that these animals require large amounts of glucose. It can hardly be maintained that injection stimulated the utilization of glucose since the blood sugar level was below normal in nearly all instances. Work is now being carried out with the purpose of de-

[5] Personal communication.

termining the fate of this disappearing sugar: whether it is oxidized, converted to fat, deposited as glycogen or changed to some other complex.

7973 C

Mechanism of Ingression in the Egg of Triturus Torosus.*

A. M. SCHECHTMAN. (Introduced by B. M. Allen.)

From the Department of Biological Sciences, University of California at Los Angeles.

In a previous paper[1] it was shown that the ventralmost surface of the zygote of the salamander, *Triturus torosus,* is carried into the interior of the egg sometime before gastrulation begins. Vitally stained marks were placed on the lower (vegetal) surface of the zygote and development allowed to proceed for 24 to 30 hours. The marks were subsequently located in the uppermost portion of the blastula floor.

In the present experiments large, clear marks were placed on the ventral (vegetal) surfaces of eggs at successive stages of cleavage. The subsequent location of the stained materials was determined by dissecting the eggs in the mid-blastula stage. Observations were

TABLE I.
Extent of Ingression of Vitally-Stained Marks Placed upon the Ventral Surfaces of Eggs at Successive Stages of Development.

Stages of Development	Presence of Stained Cells in the Floor of Blastula		
	Upper Third	Middle Third	Lower Third
Uncleaved egg (zygote)	+ (Fig. 1, A)	+	+ — in some cases*
First cleavage completed†	+ A few cells in some	+	+ — in some cases*
Second cleavage completed†	A few cells in some	+	+
Third cleavage completed†	—	+	+
Early Blastula	(Fig. 1, B)	A few cells in some	+
Later "	—	—	+

*The presence of stained material in the lower third of some eggs and its absence in others seems to be correlated roughly with the sizes of the stained marks. In several instances in which this point was observed specifically the positive cases were eggs with large stains.

†The term "completed" signifies that the cleavage furrow has reached the lower (vegetal) pole of the egg.

*This study was aided by a research grant from the University of California.

[1] Schechtman, A. M., *Univ. Calif. Publ. Zool.*, 1934, **39**, 303.

made with a device which gives a ventral or lateral view without the necessity of inverting the egg.[1] The ingression described cannot, therefore, be a result of the movement of the heavier yolk material under the influence of gravity.

The results obtained with 34 eggs are given in Table I. Typical examples are illustrated in Fig. 1. From the table it is apparent that ingression is most extensive if the stain is applied *prior* to the completion of the first 3 cleavage furrows. Stains made after this stage undergo only a relatively small degree of ingression. And finally, if stains are applied when the egg is a well-developed blastula, very little or no ingression at all is discernible.

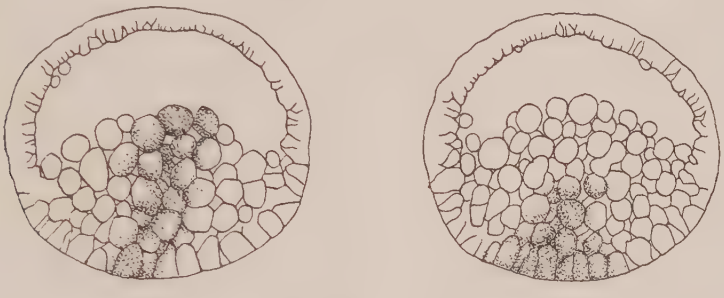


FIG. 1.

Sections through bisected blastulæ showing locations of vitally-stained materials (stippled). *A*. Egg stained in the zygote stage. *B*. Egg stained in the early blastula stage when 20-24 cells were visible from top view.

This relationship between extent of ingression and stage of cleavage gives us an insight into the mechanism involved.[2] The first 3 cleavages are the most extensive in scope, passing through a greater portion of the egg's substance. They are thus capable of displacing the stained ectoplasm to the greatest extent. Later cleavages are progressively localized to smaller and smaller portions of the egg.

These results support our original suggestion that ingression is accomplished by the movements incidental to cell-division. The displacement of the ventralmost material of the zygote to the top of the blastula floor is accomplished by the centripetal movement of the cleavage furrows.

---

2 Since this paper was sent to press the type of experiment described has been applied to the egg of the frog, *Hyla regilla*. The results were identical to those reported for *Triturus torosus*.

7974 P

Shock Durations and Measurements of Cardiac Excitability.

A. S. GILSON, JR.

From the Department of Physiology, Washington University School of Medicine, St. Louis.

A turtle heart under vagus inhibition shows a complex of functional changes. Three of these changes will be referred to here. Consider the right vagus to be stimulated repeatedly so as to produce a constant condition of inhibition in the heart. Suppose that during this period of inhibition there is a slowing of heart rate, an increase of the time required for propagation of the cardiac impulse from sinus to atrium, and a decrease in the strength of the atrial beat. Suppose that now the sinus be stimulated by shocks such as to drive the heart at a rate faster than the above, slowed, spontaneous rate but slower than the normal rate. Unless partial or complete S-A block occurs, there will be a further increase in the S-A interval and the recorded beat of the atrium will be still further depressed. In other words, the slowed, vagus-inhibited heart appears as though the time required for recovery from a given contraction has been increased at least as much as has the interval intervening between beats. Nevertheless, by the use of induction shocks of from 2 to 5 times threshold strength, "the refractory period" of the dog's auricle is reported to be shortened markedly.[1, 2]

We have studied the threshold to electrical shocks of the turtle atrium in an attempt to discover if there exist measurable changes in electrical excitability during the later part of the recovery process. Induction shocks from a Porter coil and short condenser discharges were used. A shortened absolutely refractory period was always demonstrable when the tissue was under the effect of vagus inhibition. We then chose a fixed time interval (S-I) measured between the beginning of a normal systole (S) and the time of application of a shock to elicit an interpolated systole (I). S-I was made slightly less than the normal intersystolic interval (S-S). A shock was found having a strength just sufficient to elicit the interpolated response. Vagus stimulation was then used to produce a marked inotropic depression of the atrium, sometimes with and sometimes without concomitant slowing of the heart rate. Keeping the same S-I time as in the control determinations, no significant or con-

1 Lewis, Th., Drury, A. N., and Bulger, H. A., *Heart*, 1921, **8**, 83.

2 Andrus, E. C., and Carter, E. P., *J. Exp. Med.*, 1930, **51**, 357.

sistent change in threshold to induction shocks or short condenser charges could be found.

Shocks were then produced by means of a double condenser stimulator[3] having a k value of 2 and a time to maximum voltage of 0.018 seconds. With such shocks, the absolutely refractory period was shortened during periods of vagus inhibition but for a given S-I time as above, the voltage required to stimulate the atrium was invariably increased. These results were to be expected in the light of published time-intensity curves plotted from results obtained with normal and vagus inhibited hearts under conditions of controlled rate.[4]

Such findings offer another example of the need for considering not only the intensity but also the time functions of electrical stimuli used in determining tissue excitabilities. Specifically, we would point out that when measurements are made by use of induction shocks only, there may be seen only an apparent quickened recovery of the vagus inhibited tissue following a previous systole. On the other hand, with the longer shocks which were selected to approach pararesonance with the tissue, there is demonstrable both the shortened absolute refractory period which is probably correlated with the shortened period of mechanical systole, and a later prolonged period of depression. The data obtained by the use of such long duration shocks seem to be in closer accord with the observed functional changes than are data obtained by the use of induction shocks alone.

7975 P

Fate of Hexosemonophosphate During Aerobic Recovery of Frog Muscle.*

A. H. HEGNAUER, R. E. FISHER, G. T. CORI, AND C. F. CORI.

From the Department of Pharmacology, Washington University School of Medicine, St. Louis.

An accumulation of hexosephosphate was produced in thin frog muscles (sartorius, ileofibularis, etc.) by keeping them anaerobically

[3] Monnier, A. M., *L'excitation électrique des tissues*, Paris. 1934, 115 ff.

[4] Ashman, R., and Garrey, W. E., *Am. J. Physiol.*, 1931, **98**, 109.

* Aided by a grant from the Rockefeller Foundation to Washington University for research in science.

for 2 hours in phosphate-Ringer's solution (pH 7.2) containing epinephrine in a concentration of $1:10^7$. As shown previously,[1] the hexosephosphate so formed disappears when the muscles are transferred to oxygenated Ringer's solution containing no epinephrine. In the present experiments the O_2 consumption was measured in a Warburg apparatus during the disappearance of hexosephosphate. Matched muscles were used, one group being analyzed for hexosephosphate and lactic acid at the beginning and the other at the end of the aerobic period. The O_2 consumption was markedly increased in the first 20 minutes and then returned slowly to a steady rate, which was assumed to represent the basal O_2 consumption. The total period of O_2 measurement was between 1.5 and 2 hours. In one group of experiments the muscles were poisoned with iodoacetate (1:10,000) during the last half hour of the anaerobic period in order to investigate the disappearance of hexosephosphate in muscles with inhibited lactic acid formation.

The following results (in mg. per 100 gm. muscle) were obtained (average of 4 experiments each).

TABLE I.

	——Decrease in——		Carbohydrate equivalent of	
	Hexose-phosphate	Lactic acid	Total O_2 consumption	Extra O_2 consumption
Without	41	7	16	7
With iodoacetate	42	4	14	2

It may be seen that hexosemonophosphate disappears aerobically at the same rate whether or not the path to lactic acid is blocked by iodoacetate. In either case the total O_2 consumption is insufficient to account for the disappearance of hexosephosphate and the extra O_2 consumption accounts for only 1/6 to 1/20 of the amount which disappears. This seemed to indicate that hexosephosphate is disposed of in some other way and suggested the possibility that it might be reconverted to glycogen.

The following experiments were performed on summer frogs because of their low glycogen content. An increase in hexosephosphate was produced by tetanic stimulation through the pelvic nerves. Small muscles of the right and left side were dissected immediately after stimulation. When the muscles of the 2 sides were analyzed at once, the differences in glycogen were: +40, +24, —26, +30, —11, —38, average +3 mg. %. When the muscles of one side were analyzed at once and those of the other side after 2

[1] Cori, G. T., and Cori, C. F., *J. Biol. Chem.*, 1934, **107**, 5.

hours of recovery in O_2, the increases in glycogen were: +125, +110, —60, +154, +75, +66, +77, +42, +71, average +73 mg. %. The average amount of lactic acid which disappeared during 2 hours of recovery in O_2 was 38 mg. %, hence more glycogen was resynthesized than could have come from lactic acid. Assuming that ¼ of the lactic acid which disappeared was oxidized, while the rest was reconverted to glycogen, there remain $73 - 29 = 44$ mg. % of glycogen which could have been formed from hexosephosphate. The amount of hexosephosphate which disappeared during 2 hours of recovery in O_2 was more than sufficient to account for this increase in glycogen.

In order to determine the disappearance of hexosephosphate after iodoacetate poisoning, previously pithed frogs were first tetanized through the pelvic nerves and were then given an intravenous injection of iodoacetate.† During 1 hour of recovery in O_2 the muscles poisoned with iodoacetate lost on an average, 46 mg. % hexosephosphate, as compared to 50 mg. % for unpoisoned muscle. The fact that hexosephosphate disappears from muscle poisoned with iodoacetate suggests that resynthesis to glycogen occurs directly and not by way of lactic acid.‡

Summary. More hexosemonophosphate disappears during aerobic recovery of frog muscle, either unpoisoned or poisoned with iodoacetate, than is accounted for by the total oxygen consumption. Glycogen determinations show that hexosemonophosphate is resynthesized to glycogen.

† Control experiments showed that muscles removed 5 minutes after injection were poisoned completely, since no lactic acid was formed during rigor. Hexosemonophosphate and diphosphate do not accumulate to an appreciable extent in muscles poisoned with iodoacetate until rigor begins to develop. In the above experiments, the muscles were analyzed before the onset of rigor.

‡ The first step in the resynthesis to glycogen is presumably the splitting off of the phosphoric acid group. The fermentable sugar content of muscle was found to increase when previously stimulated muscle was allowed to recover in O_2.

7976 P

Influence on the Electrocardiogram of Changing Electrical Axis from Anterior to Posterior Mediastinum.

J. R. SMITH, M. PRINZMETAL, AND W. B. KOUNTZ. (Introduced by D. P. Barr.)

From Washington University School of Medicine.

The influence of position of the heart on the electrocardiogram has in recent years received considerable attention. That position of the electrical axis may be of importance was first emphasized by Lewis,[3] and the subsequent experiments of Katz and Barker[1, 2] have indicated the importance of a more detailed study of the subject.

Kountz[4] has shown that placing hearts of dogs within the human pericardial cavity yield curves of a similar nature to those obtained in man. This is especially true when the dog heart has the same interthoracic relationship as that of man. Changing the position of the electrical axis will greatly influence the electrocardiographic curves. This observer has further shown that the dog's heart, readily movable from anterior to posterior mediastinum, yields markedly dissimilar curves when the position of the heart is altered anteriorly or posteriorly. These experiments have served to emphasize the importance of further investigation along these lines.

A dog heart-lung preparation was set up (Starling), and the heart and lungs removed from its body; the thorax of a cadaver, recently dead was opened anteriorly. The esophagus of the heart-lung preparation was connected by a wire to an electrode, which could be placed in any part of the human thorax. One chest electrode was placed in the left anterior chest wall; another in the right posterior wall, care being taken that these electrodes were equidistant from the midline of the chest. There were thus formed 2 vectors, one lying anteriorly, and the other posteriorly. The apex of the heart-lung preparation was connected to the esophagus in the chest. Finally, the 3 leads of the electrocardiograph were connected to the arms and left leg of the cadaver, by embedding the electrodes in the muscles. By this arrangement it was found that a cardio-

1 Katz, L. N., and Ackerman, W., *J. Clin. Invest.*, 1932, **11**, 12.

2 Barker, P. S., MacLeod, A. G., and Alexander, J., *Am. Heart J.*, 1930, **5**, 720.

3 Lewis, T., *Mechanism and Graphic Registration of the Heart Beat*, London, 3d edition, 1925.

4 Kountz, W. B., to be published.

electric impulse arising in the heart-lung preparation was reflected in the electrocardiograms obtained from the cadaver (the heart-lung preparation being outside the human body), and that, by changing the chest electrode in contact, the different vectors, representing electrical axes, could be obtained.

With the chest electrode in the left anterior chest, extrasystoles were produced by stimulation of the lateral wall of the left ventricle of the heart-lung preparation. The extrasystole curves thus produced were down in lead 1, and up in lead 3. Stimulation of the lateral wall of the right ventricle of the heart-lung preparation resulted in the opposite, with extrasystole complexes upward in lead 1 and downward in lead 3. These results were similar to those obtained by Barker[2] in man.

With the chest electrode in the right posterior chest, extrasystoles arising in the lateral wall of the left ventricle of the heart-lung preparation yielded curves upward in lead 1 and downward in lead 3; those arising in the lateral wall of the right ventricle of the heart preparation gave complexes downward in lead 1 and upward in lead 3. The results here were similar to those obtained by Lewis in the dog. It is seen that here there is a complete reversal of curves, as when the chest electrode was in the left anterior chest.

Since the chest electrodes formed identical angles with the midline of the body, though one was anteriorly and the other posteriorly placed, one might have expected the same type of electrocardiogram with the chest electrodes in the anterior or posterior chest. This, however, was not found. It would seem, therefore, that the factor of contact and electrical resistance of tissue, besides position of the electrical axis, would be an important influence in determining the type of electrocardiographic curve of extrasystoles arising in the heart.

7977 P

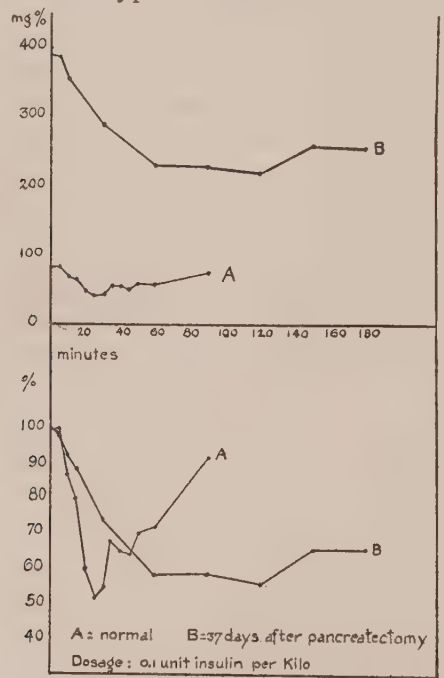
Blood Sugar Curves in Normal and Diabetic Dogs After Intravenous Injection of Insulin.*

B. N. BERG, J. GROSS, J. McAFEE, AND T. F. ZUCKER.

From the Department of Pathology, College of Physicians and Surgeons, Columbia University.

The following report deals with the blood sugar curve after the intravenous injection of insulin into normal and depancreatized dogs. The study includes over 50 observations upon normal and 350 observations upon diabetic animals.

Experiments on blood sugar lowering were performed on male dogs weighing 12 to 18 kilos, 18 hours after the last (afternoon) feeding and routine insulin injection. Doses from .02 to 1.0 units of insulin (Lilly) per kilo given in a volume of 1 cc. were injected into the jugular vein. Glucose determinations on tungstic acid filtrates were made by the Shaffer-Hartman method. The time intervals shown in the chart were found to bring out most clearly the differences in the 2 types of curves.



*This work was aided by a grant from the Eli Lilly Company and we also wish to acknowledge their liberal supplies of insulin.

The chart gives typical examples of time curves after the injection of 0.1 unit of insulin per kilo intravenously before and after removal of the pancreas. The upper curves show the results expressed in mg. % of blood sugar. The striking change in amount of sugar transported from blood to tissues by a given dose of insulin is apparent. The conclusion that in the diabetic the activity of insulin is enhanced is shown to be erroneous when we compare relative blood sugar lowering on the basis of the fraction removed, assigning the value of 100% to each initial blood sugar level (Scott[1]). This is shown in the lower set of curves.

In the normal dog the blood sugar falls rapidly, reaching its lowest level within 20 to 30 minutes after the injection of insulin. This is followed by an abrupt rise and then restoration to approximately the original value within 90 minutes. The return phase is usually represented by a broken curve frequently giving several maxima and minima. Duration of action, varying with the dose of insulin, reflects itself in the return phase but never in the initial drop which terminates sharply at about 25 minutes with all doses so far observed.

In the diabetic animal, although the fraction of blood sugar removed may be about the same, the rate of initial action is distinctly less than in the normal. In the smaller dose range (up to about 0.3 units per kg.) there is an uninterrupted drop lasting one hour or more and according to the condition of the animal, reaching levels of 40 to 80% of the initial value. This is followed by a gradual return to the original level occupying at least another hour. With larger doses the continuous blood sugar lowering may be extended for over 2 hours, coming within range of the level of non-fermentable reducing substance, and followed by a correspondingly slow recovery. With no dose and at no time is there any evidence of the sharp upturn which is characteristic of the insulin response in the normal. The "diabetic type" of curve persists in animals examined up to one year after pancreatectomy. The character of the response is unaffected by the level of the initial blood sugar.

Instances of similar characteristic curves after intravenous administration of insulin in normal and diabetic man have been reported.[2-7] Bøggild[8] has also described the course of action of intravenous insulin in normal dogs.

[1] Scott, E. L., and Dotti, L. B., *Arch. Int. Med.*, 1932, **50**, 511.

[2] Lyman, R. S., Nicholls, E., and McCann, W. S., *J. Pharm. and Exp. Therap.*, 1923, **21**, 343.

[3] Raab, W., *Z. f. d. ges. exp. med.*, 1924, **42**, 723.

In order to determine how soon after removal of the pancreas the change from the normal to the diabetic type of curve occurs the following experiment was performed. After establishing the normal response of the blood sugar to the intravenous injection of insulin the pancreas was removed from a dog and observations were made at the following intervals after pancreatectomy: 18, 42, 66, 90, 138 hours and 14 days. The dose of insulin was 0.1 unit per kilo.

Eighteen hours after removal of the pancreas the blood sugar was 311 mg. %. The shape of the blood sugar curve deviated markedly from the normal and had many of the characteristics of the diabetic. Compared with the curves obtained before pancreatectomy, the initial rate of decrease in blood sugar was diminished, minimum sugar values were reached later than in the normal, and the abrupt upturn was largely lost, its place being taken by a slow gradual return. However, the curve did not assume the form of an uninterrupted drop until 138 hours after pancreatectomy. The deviations at 25 minutes decreased gradually in consecutive observations. Comparison of the percentage reduction of blood sugar after 14 days with the results obtained at 138 hours showed a marked increase in sensitivity to insulin.

Apparently a number of factors are affected by the removal of the pancreas. Although the blood sugar level indicates a fully developed diabetes soon after removal of the pancreas, the adjustment of the diabetic response to insulin is not completed until several days later. Experiments on a number of animals with varying time intervals have concordantly shown the transition from the normal to the diabetic type of curve as outlined above.

---

[4] Bodansky, A., and Simpson, S., *Quart. J. Exp. Physiol.*, 1927, **17**, 57.

[5] Csépai, K., and Ernst, Z., *Wien. Klin. Wchnschr.*, 1928, **41**, 25.

[6] Norgaard, A., and Thaysen, T. E. H., *Act. Med. Scand.*, 1929, **72**, 492; Thaysen, T. E. H., *Act. Med. Scand.*, 1930, **73**, 408.

[7] Collens, W. J., and Grayzel, H. G., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 487.

[8] Bøggild, D. H., *Acta Med. Scand.*, 1933, **79**, 458.

7978 C

Prevention of Poliomyelitis in Monkeys by Means of Hyperpyrexia.*

HEINRICH F. WOLF. (Introduced by Maurice Brodie.)

The remarkable results of hyperpyrexia in various infectious diseases, particularly in gonorrheal infections, has turned the attention to its application in a great variety of disorders. The fact that the gonococcus can be destroyed *in vivo* as well as *in vitro* by temperatures compatible with life led me to the assumption that the same process may be effected in the treatment of virus diseases.

I concentrated my experiments on the treatment of poliomyelitis in monkeys and the object of this paper is to show that it is possible to prevent the development of poliomyelitis in monkeys completely or to affect the disease in such a way that the symptoms are only slight and disappear rapidly, if monkeys are treated immediately after inoculation, before symptoms have had time to appear.

The monkeys treated were inoculated by Dr. Maurice Brodie intracerebrally according to the technique and the system of infective doses worked out by him.

The hyperpyrexia was produced by a short wave apparatus operated by a spark gap and producing a wave of 16 meters. The electrodes consisted of 2 metal plates 6x8 inches placed side by side and covered with heavy cardboard. On this cardboard a cushion of sponge rubber was placed which assures an air space between the electrodes and the animal of about ¾ of an inch. The electrical field passes from one electrode to the other through the body of the monkey which is lying on the rubber cushion. After various trials the best method was found to be to tie the monkey to a board on its back and placing the board on the electrodes. The amount of field energy was about 2,000 M.A.

The rectal temperature of the monkey rises from normal, that is 101° to 103.4° to 107° and 108° within half an hour. Once the temperature of 108° to 108.6° is reached it is necessary to interrupt

*The experiments were done on a grant of the Council of Physical Therapy of the A.M.A. It gives me great pleasure to express my sincere gratitude to Dr. William Park of the Bureau of Laboratories, Board of Health, New York City, for permission to work there and to Dr. Maurice Brodie for the necessary help. I fully realize that it would have been impossible for me to undertake such investigations if Dr. Brodie had not placed his experience and his cooperation at my disposal. I also wish to express my thanks to Mr. Egbert von Lepel who constructed the short wave apparatus and assisted in my first experiments.

the treatment and resume it when the temperature has fallen to about 107°. The rise of temperature from 107° to 108° or more may be accomplished in 1 to 1½ minutes. It is, therefore, necessary to measure the temperature every minute or one and a half minutes when the current is on, so that the temperature may not rise above the tolerable limit, and every 5 minutes while the current is turned off, in order to forestall a great drop in temperature.

Monkey No. 650 received 10 M.C.P. on March 22nd; treated on the 24th, 25th and 27th of March. The animal received hyperpyrexia sustained above 107° for 4½ hours. The maximum temperature was 109° for a few minutes. The monkey did not develop any signs of disease.

Monkey No. 651, infected on the same day, March 22nd, with the same dose. Treated on March 24th, 26th and 27th. Hyperpyrexia with heat sustained above 107° for 3 hours. The maximum temperature was 109.5°. The animal did not develop any signs of the disease.

The control monkey, No. 647, infected with only 1 M.C.P. became prostrate on the 8th day.

Monkey No. 640, inoculated May 23rd with 10 M.C.P. Treated on May 25th, 27th, 28th, and 30th, hyperpyrexia above 107° sustained for about 3½ hours, total treatment given 7½ hours. The monkey showed a slight weakness of the right hand but recovered completely.

Control monkey No. 756 received between 1 and 2 M.C.P. and was prostrate after 5 days.

Monkey No. W-2, infected October 26th with 5 M.C.P. Treatment started October 27th and continued October 28th, 31st, November 3rd. Temperature sustained above 107° for more than 4 hours. The monkey showed no signs of disease.

Control monkey No. W-3, infected on the same day with the same dose became paralyzed and died October 31st.

Control monkey No. W-1, infected on the same day as W-2, and W-3, with the same dose. Treated first October 30th, temperature already 105.2°, second treatment October 31st, third treatment November 1st, received sustained heat of over 107° for about 4 hours. Showed slight facial palsy on November 1st, became paralyzed and died November 3rd.

Monkey No. 821, infected with 5 M.C.P. October 7th. Treated October 8th, 9th and 10th. On the third day of treatment the monkey showed severe diarrhea and general weakness and the treatment was discontinued. The monkey improved and no more treatments

TABLE I.
Treatment in Incubation Period with Controls.

Number	Date of Infect.	M.C.P.	Treatment No. of Days after Inoculation Incubation Stage	Preparalytic Stage	Duration of Treatment above Temp. of 107; Maximum Temp.	Outcome	Remarks
650	3/22	10	2 3 5		4½ hrs. in all; maxim. temp. 109	remained well	
651	3/22	10	2 4 5		3 hrs., maxim. temp. 109.5	,, ,,	
Control	3/22	1				died 7th day	
821	10/7	5	1 2 3*		3 hrs., maxim. 109	prostrate on 15th day	*Strong diarrhea, weak, temp. to 105
Control	10/7	3				prostrate 7th day	
W-2	10/26	5	1 3 6 9		6 hrs., maxim. temp. 108.6	remained well	
Control	10/26	5				prostrate 5th day	
,,	10/26	5		5 6 7	5 hrs., maxim. temp. 108	prostrate on 9th day	Showed slight signs of paralysis on 7th day
640	5/23	10	2 4 5 6		3½ hrs., total treatment 7½ hrs.	remained well	Right hand weak on 8th day, recovered
Control	5/23	1-2				died 8th day	

were given as no signs of poliomyelitis appeared. The monkey seemed perfectly well but suddenly became paralyzed on the 14th day. This greatly prolonged incubation period indicates that in view of the previous experience the animal could probably have been saved if the treatments had been continued.

The control monkey received 3 M.C.P. October 7th, was treated once on October 15th, body temperature 104.2°. Paralyzed on the next day.

To summarize the above experiments: Five animals were used in this series for experimental purposes and 5 for controls. Four monkeys were infected intracerebrally with 5 and 10 M.C.P., respectively, and treated 1 or 2 days after inoculation. None of these became paralyzed. One monkey showed slight weakness of one arm and recovered completely.

The control monkeys which were not treated at all or treated in the preparalytic stage *after the onset of fever* became prostrate. One which was treated energetically (No. W-3) showed a prolonged preparalytic stage.

One monkey which was infected with 10 M.C.P. and treated 1 and 2 days after inoculation in which the treatment had to be interrupted on account of diarrhea became prostrate on the 14th day. The control monkey which received only 3 M.C.P. and was given only one treatment on the 6th day (in the preparalytic stage) was prostrate on the 7th day. (Table I.)

From these experiments it seems to be definitely established that it is possible to abort the development of poliomyelitis in monkeys by hyperpyrexia if the treatments are given soon (1 or 2 days) after the inoculation and if they are continued over a sufficiently long time. The uniformity of results permits their publication in spite of the relatively small number of experiments.

In the following I should like to present a hypothesis for the action of hyperpyrexia. The assumption that its efficacy is due to a viruscidal action is obviously erroneous. If this were so we should expect results regardless of intensity of infection and the time elapsed between inoculation and treatment. It occurred to me that the unquestionable action might be explained in the following way:

The virus of poliomyelitis does not develop if the monkey is injected intravenously, obviously because it can not pass the blood-brain barrier and is destroyed in the blood. On the other hand if injected into the brain or nose it can exert its action on the nerve cells because the antibodies in the blood can not pass the barrier and destroy the virus. It may be possible that hyperpyrexia by dilation

of the capillaries makes the blood-brain barrier permeable to some extent for the antibodies and if these can attack the virus early in the incubation period they may neutralize the virus. Once the virus has had a chance to multiply and produce a reaction in the body in form of fever no results were obtained from hyperpyrexia if large infective doses were injected. Experiments not yet concluded on monkeys infected with small doses indicate that the size of initial dose is of great importance.

7979 P

Estrogenic Dihydroxy Compounds in the Urine of Pregnant Mares.

OSKAR WINTERSTEINER, ERWIN SCHWENK, AND BRADLEY WHITMAN. (Introduced by H. T. Clarke.)

From the Department of Biological Chemistry, College of Physicians and Surgeons, Columbia University, and the Research Laboratories of the Schering Corporation (Bloomfield, N. J.)

Schwenk and Hildebrandt[1] described the isolation from the urine of pregnant mares of a new compound of high estrogenic potency. This substance, designated by them δ-follicular hormone, melted at 209°, gave a characteristic purple color when coupled with p-nitrodiazobenzene and showed a gold yellow fluorescence in concentrated sulfuric acid solution. Analysis of the compound itself and of its mono-benzoate indicated the composition $C_{18}H_{22}O_2$. Treatment with ketone reagents failed to give characteristic derivatives.

Another batch of pregnant mare's urine has now been worked up in the laboratories of the Schering Corporation (Bloomfield, N. J.). The compound present in largest amount in the crude fraction containing the phenol-alcohols is the above δ-follicular hormone. Analysis of the present preparation gave figures agreeing better with $C_{18}H_{24}O_2$ than with $C_{18}H_{22}O_2$. The preparation of a di-p-nitrobenzoate (m.p. 260° uncorr.) leaves no doubt that both oxygen atoms are present in the form of hydroxyl groups.

A second substance isolated is apparently identical with the lower melting member (m.p. 174° uncorr.) of the pair of isomeric hydroxyphenols ("α-dihydrofollicular hormone") which Schwenk and Hildebrandt[2] obtained by reduction of theelin. It shows the same

1 Schwenk, E., and Hildebrandt, F., *Naturwis.*, 1932, **20**, 658.

2 Schwenk, E., and Hildebrandt, F., *Naturwis.*, 1933, **21**, 177.

characteristic blue fluorescence in sulfuric acid solution as the dihydrofollicular hormone and gives no depression of melting point when mixed with the latter. Furthermore, a small amount of a compound melting at 236° (uncorr.) which is more difficultly soluble in absolute methyl alcohol than the δ-hormone could be separated from the top fractions of the latter. The amount of the 2 last-named substances isolated was not sufficient for analysis and the preparation of derivatives.

The above findings establish for the first time the occurrence of estrogenic dihydroxy compounds in the urine of pregnant mares. Schwenk and Hildebrandt[2] have suggested that the dihydro-follicular hormone, which is about 6 times as active as theelin, may be the estrogenic substance actually circulating in the body fluids.* In this connection it may be pointed out that the only estrogenic substance which has been isolated in pure form from a mammalian organ, the emmenin obtained by Collip and his collaborators[3] from the placenta, has been shown by Butenandt and Browne[4] to be identical with theelol, the non-ketonic trihydroxy compound also present in human pregnancy urine. It is also conceivable that the very high degree of estrogenic potency reported by Zondek[5] for stallion's urine is at least partly due to the presence of substances of purely alcoholic character.

7980 P

Urinary Excretion of Vitamin C in Pneumonia.

E. HARDE, I. A. ROTHSTEIN, AND H. D. RATISH. (Introduced by J. G. M Bullowa.)

From the Littauer Pneumonia Research Fund, New York University, Harlem Hospital Station.

In previous work[1] on the tissues of laboratory animals we have

* The recent announcement of MacCorquodale, Thayer and Doisy at the 29th Annual Meeting of the American Society of Biological Chemists in Detroit, April 13, 1935, of the isolation from follicular fluid of dihydrotheelin (m.p. 173°, corr.) furnishes additional evidence for the correctness of this viewpoint.

3 Collip, J. B., *Can. Med. Ass. J.*, 1930, **22**, 212, 215, 761; Browne, J. S. L., *Can. J. Res.*, 1933, **8**, 180.

4 Butenandt, A., and Browne, J. S. L., *Z. physiol. Chem.*, 1933, **216**, 49.

5 Zondek, B., *Nature*, 1934, **133**, 209.

1 Harde, E., *C. R. de l'acad. des Sc.*, 1934, **199**, 618; Harde, E., and Philippe, *C. R. de l'acad. des. Sc.*, 1934, **199**, 738; Harde, E., and Benjamin, H. R., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 651.

found the vitamin C content to be reduced in many infections and intoxications. This suggested that cevitamic acid acted not only as an oxidative factor, in normal respiratory processes—but also had a neutralizing rôle in various pathological conditions other than scurvy.

Yavorsky, Almaden and King[2] examined human tissues from autopsy for their vitamin C content, and noted that generalized infections were more common among those having a low vitamin C content in their tissues. Worringer and Sala[3] reported scurvy in infants following diphtheria and pertussis.

Ten cases of pneumonia were examined on the Pneumonia Service of Doctor Bullowa at Harlem Hospital. The method of Hess and Benjamin,[4] and Birch, Harris and Ray[5] was followed using the dye 2,6,dichlorophenolindophenol. These authors noted that in normal individuals after the ingestion of large doses of vitamin C it was rapidly eliminated in great quantities in the urine. Recently Harris and Ray[6] found that when vitamin C was low in the diet individuals tend to excrete less vitamin C in urine than well-nourished ones. However, more reliable results as to the state of vitamin C saturation or unsaturation of the tissues can be obtained by examining the urinary excretion after administering large test doses of the vitamin. A normal excretion for adults per day the authors estimate as 15-30 mg.

The technique of Harris and Ray was followed as closely as possible. Occasionally, however, it has been impossible to get every specimen of urine in the 24 hours, and this has been noted in our calculations. Of the 10 cases 5 were given no saturation test.* Two of these were fatal cases. In the first, (a pneumococcus type I† pneumonia with empyema) in 3 specimens of urine voided 12 hours before death 8.6 mg. of cevitamic acid was excreted suggesting a normal output. In the second fatal case there was a lower excretion, 5 mg. in 20 hours. The calculation was inexact, a highly colored urine made the end point very difficult to determine.

2 Yavorsky, M., Almaden, P., and King, C. G., *J. Biol. Chem.*, 1934, **106**, 525.

3 Worringer, J., and Sala, A., *Rev. Franc. de Pediat.*, 1928, **33**, 806.

4 Hess, A. F., and Benjamin, H. R., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 855.

5 Birch, T. W., Harris, and Ray, *Biochem. J.*, 1933, **27**, 590.

6 Harris, L. J., and Ray, S. N., *Lancet*, 1935, 228.

* For the saturation test the patients were given large quantities of orange juice or pure cevitamic acid. This was generously supplied to us by Merck & Co. and Hoffman-LaRoche.

† In these cases we have found no definite correlation with the types of pneumococcus causing the disease, but the present data is insufficient.

In the 3 other cases, one excreted a normal amount, 30 mg. in 24 hours. In the second case a Pneumococcus type 3 complicated by a *Streptococcus beta* in the blood, the titration was so low as to be inexact, 1.9 mg. in 24 hours, (one specimen lost). Seven days later the patient was recovering and the amount excreted was 7 mg. in 12 hours. In the third case the excretion was 11 mg. in 24 hours only slightly lower than normal.

In 5 other cases saturation tests were made. One, a fatal case, 2 days before death had a normal content 13 mg. in 24 hours, 1 specimen missing. Given 350 mg. of cevitamic acid in the next 24 hours, resulted in no high peak of excretion—only 21 mg. excreted, the immediate "saturation" test being negative.

In the 4 other cases, one gave normal content over 12-hour period. He was "saturated" for 2½ days and then examined. Excretion was normal, 30.2 mg. per 24 hours, with no immediate saturation. Second case, recovering, in 12 hours only 5 mg. excreted. After 48 hours of hypervitamin C feeding saturation test was negative, 8 mg. in 15 hours. The 3rd case gave similar results. No exact calculation was possible, as a complete 24-hour specimen was not obtained. The urine titrated showed persistently low content. After 24-hour "saturation" no saturation had occurred.

Case 4. Examinations were commenced on the 19th day of the illness. Patient had been given a great deal of orange juice. The first titrations were but slightly below normal. After "saturation" for 48 hours there was no increase, 12.3 mg. Then 400 mg. of vitamin C were given—excretion was 14.10 mg. After continued saturation, 14.16 mg. excreted. It was only on the tenth day of the saturation that an overflow occurred—59.35 mg. was excreted in 10 hours. One sample was lost. In the next 6 hours the excretion was 36 mg., making a total in 16 hours of 95 mg. The patient was at this time recovering.

We have thus found in accord with Harris and Ray that by the saturation test a hypovitaminosis may be shown even though the titration of the urine alone might give normal values. Whether the deficiencies in the vitamin we have found in certain cases are due to the previous diet of the patients or to the intoxication of the pneumonia or to both factors we have not yet determined. We also note that certain of the lesions of the cardiovascular system in pneumonia suggest those found in scorbutic conditions.

The clinical results will be reported later.

7981 P

Effect of Splenectomy on Production of Cobalt Polycythemia.*

JAMES M. ORTEN.†

From the Department of Physiological Chemistry, Yale University.

Berwald, Arseneau, and Dooley[1] reported the preliminary results of an experiment to determine the effect of splenectomy on the production of cobalt polycythemia. Although no direct statement of the conclusions derived from their data was made, one gains the impression that the removal of the spleen of the adult rat prevents the rise in erythrocytes obtained in normal animals after cobalt administration. Such an impression, however, is not justified in view of the incompleteness of the data presented. Attention is also directed to the fact that these authors refer incorrectly to an article by Orten, Underhill, Mugrage, and Lewis[2] as having "ashed the organs, finding the largest amounts of cobalt in the liver, pancreas and spleen, and minute quantities in the bone marrow." The paper referred to contained no data of this kind.

In the present communication are presented the preliminary results of an experiment to determine the effect of splenectomy on the hematopoietic potency of cobalt. Male albino rats weighing from 40 to 50 gm. at weaning (21 days of age) were placed on a diet consisting of dried, whole cow's milk (Klim) supplemented by 0.5 mg. of iron as purified ferric chloride and 0.025 mg. of copper as copper sulfate daily. At 25 days of age, the animals were divided into 3 groups. One group (10 rats) was continued on the milk-iron-copper diet as controls. Laparotomies and splenectomies were performed on the animals of the other 2 groups of 12 and 5 rats, respectively, and they were continued on the basal, milk-iron-copper ration; when they were 40 days of age, cobalt feeding, at a level of 0.5 mg. of cobalt as cobalt chloride daily, was started. Weekly weight and bi-weekly erythrocyte and hemoglobin determinations were made by procedures previously described.[3]

*Aided by a grant from the research funds of the Yale University School of Medicine, 1933.

† Alexander Brown Coxe Fellow in Physiological Chemistry, Yale University, 1932-33.

1 Berwald, W. P. E., Arseneau, J. H., and Dooley, M. S., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 420.

2 Orten, J. M., Underhill, F. A., Mugrage, E. R., and Lewis, R. C., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 174.

3 Orten, J. M., and Smith, A. H., *Am. J. Physiol.*, 1934, **108**, 66.

TABLE I.
Hemoglobin and Erythrocytes in the Blood of Control and Splenectomized Rats.

Weeks on	Milk-Fe-Cu Control				Milk-Fe-Cu-Co Control				Milk-Fe-Cu-Co Splenectomized			
	Hemoglobin gm./100 cc.		Erythrocytes M/cmm.		Hemoglobin gm./100 cc.		Erythrocytes M/cmm.		Hemoglobin gm./100 cc.		Erythrocytes M/cmm.	
Diet	Ave.	Range	Ave.	Range	Ave.	Range	Ave.	Range	Ave.	Range	Ave.	Range
0	10.6	8.3-13.2	6.5	5.5-7.2	10.5	8.1-12.3	6.3	5.4-7.6	10.2	9.3-11.6	6.3	6.0-6.8
2	13.1	11.3-14.6	6.7	5.4-8.1	14.4	11.4-16.3	7.1	4.9-8.3	10.6	7.2-14.3	4.4	2.5-7.5
4	13.1	11.6-14.5	7.1	5.1-8.6	17.3	16.3-18.9	8.4	7.5-10.1	15.2	11.7-18.8	6.4	3.8-9.7
6	13.9	12.5-15.6	7.7	6.7-8.8	18.5	15.9-21.6	9.8	8.1-11.3	17.2	16.4-18.0	8.2	6.3-9.9
8	14.2	13.5-15.6	7.7	6.6-8.6	18.7	16.4-20.7	9.7	8.5-10.4	17.8	16.2-19.1	9.0	7.5-9.9
10-12	14.7	13.5-15.8	8.4	7.4-8.9	20.2	16.6-24.2	10.2	8.5-12.3	17.4	16.8-17.9	9.9	8.6-11.1
20	13.2	12.9-13.5	8.0	7.0-8.9	18.8	15.7-22.0	10.7	9.3-12.7	18.1	17.3-19.8	10.1	9.0-11.1

The average and minimum and maximum hemoglobin and erythrocyte values obtained on the animals of the various groups for a 20-week period are given in the accompanying table. During the entire experiment, the erythrocyte and pigment values of the milk-iron-copper controls were within the lower limits found in normal stock rats of the same age,[3] whereas those of the cobalt-treated, laparotomy controls showed the characteristic increase to high levels. During the first few weeks of the experiment, 3 of the cobalt-treated, splenectomized animals developed a severe anemia; subsequently, however, the erythrocyte and hemoglobin values of these animals, like those of the other members of the group, slowly increased and finally attained levels decidedly higher than those of the untreated controls and differing but little from those of the cobalt-treated, laparotomy controls.

It is possible that the marked decrease in erythrocytes and pigment observed during the early part of the experiment in several of the splenectomized animals may have been a manifestation of "Bartonella anemia," inasmuch as small bodies, resembling "Bartonella bodies,"[4] were found in the erythrocytes of blood smears stained with Giemsa's stain. Further studies are planned to determine the possible effect of Bartonella infection on the results herein reported.

Conclusion. From the foregoing data, the tentative conclusion is drawn that the removal of the spleen of the young rat may delay but does not prevent the production of polycythemia by cobalt.

7982 P

Relation of Calcium to Blood Formation.*

JAMES M. ORTEN,† ARTHUR H. SMITH, AND LAFAYETTE B. MENDEL.

From the Department of Physiological Chemistry, Yale University.

Previous investigations in this laboratory have shown that young albino rats maintained on a diet deficient in inorganic salts fail to grow normally and develop unique hematological abnormalities, including a marked polycythemia and a concurrent, chronic anemia.[1, 2]

4 Ford, W. W., and Eliot, C. P., *J. Exp. Med.*, 1928, **48**, 475.

* Aided by a grant-in-aid, National Research Council, 1934.

† National Research Council Fellow in Medicine, 1933-34; Alexander Brown Coxe Fellow, Yale University, 1934-35.

1 Swanson, P. P., and Smith, A. H., *J. Biol. Chem.*, 1932, **98**, 479.

2 Orten, J. M., and Smith, A. H., *J. Biol. Chem.*, 1934, **105**, 181.

Analyses of the diet[3] have demonstrated that it is extremely low in calcium, sodium and chloride, and deficient in potassium, magnesium, phosphorus, and possibly iron.[4] Inasmuch as a lack of calcium appeared to be the most serious inorganic deficiency in the ration, a study was made of the hematological effects produced by the addition of this element to the low-ash ration.

The effect of calcium was investigated by both the curative and preventive procedures in uniform, vigorous, young male albino rats, selected and cared for as described previously.[2] In the curative procedure, the animals were placed on the basal low-ash ration[2] for an 8-week preliminary period and then were given calcium carbonate at a level of 50 mg. of calcium daily, the amount consumed by comparable normal controls. During the experimental period, the basal low-ash ration was fed in that daily amount ingested by the animal during the final 2 weeks of the preliminary period. In the preventive procedure, the young rats were placed directly on the calcium-supplemented, low-ash ration. The amount of the basal low-ash diet fed was restricted to that daily quantity ingested by unsupplemented controls. Simultaneously, 2 types of control animals were studied for each calcium-supplemented group: (a) unsupplemented controls given the low-salt diet; (b) inanition controls fed the same daily amount of the basal low-ash ration as the unsupplemented controls but containing, in addition, the quantity of salt mixture ingested daily by normal animals of the same weight. Adequate amounts of the vitamins[2] were supplied daily to all animals in equal quantities. Redistilled water was provided *ad libitum*. Each group consisted of at least 10 animals. Body weights were followed weekly; erythrocyte and hemoglobin determinations were made bi-weekly by procedures previously described.[5]

In the curative series of experiments (Table I), it was found that the animals of the inanition control group soon attained normal erythrocyte and hemoglobin levels,[5] whereas the unsupplemented control rats continued to show the typical polycythemia with a chronic anemia. The calcium-supplemented animals also showed a progressive decrease in erythrocytes to a normal value and an increase in pigment toward normal. Similar results were obtained in the series studied by the preventive procedure (Table I). The erythrocyte counts and pigment concentrations of the calcium-supplemented animals compared favorably with those of the inanition control group throughout the entire experimental period.

---

[3] Smith, A. H., and Smith, P. K., *J. Biol. Chem.*, 1934, **107**, 681.

[4] Brooke, R. O., personal communication.

[5] Orten, J. M., and Smith, A. H., *Am. J. Physiol.*, 1934, **108**, 66.

TABLE I.
Average Hemoglobin and Erythrocyte Values for Control and Calcium-Fed Rats.

Weeks of Exper.	Inanition Controls R.B.C. M. per cmm.	Hb gm. per 100 cc.	Low-Salt Controls R.B.C. M. per cmm.	Hb Gm. per 100 cc.	Ca Supplemented R.B.C. M. per cmm.	Hb Gm. per 100 cc.
			Curative Procedure.			
0	10.63	12.2	10.85	12.7	10.62	13.4
2	9.69	13.2	10.53	12.2	10.68	14.6
4	9.82	14.5	9.91	12.1	9.03	14.8
6	9.77	15.2	9.72	11.4	8.84	15.2
8	8.74	15.5	9.77	11.6	8.77	15.7
10	8.77	15.7	10.07	12.3	8.22	15.3
			Preventive Procedure.			
0	6.43	12.9	6.58	12.3	5.89	11.9
2	7.83	14.3	8.44	13.6	8.09	15.9
4	8.63	15.5	9.57	13.3	8.89	16.4
6	8.79	15.8	10.68	13.0	9.26	16.7
8	8.72	16.0	10.64	12.3	9.38	16.4
10	9.20	16.1	10.53	12.2	9.49	16.8
12	8.98	16.1	9.91	12.1	9.01	17.1

The foregoing results permit the conclusion that the presence of calcium in the diet deficient in certain other inorganic elements both cures and prevents the development of the expected polycythemia and concomitant chronic anemia.

The significance of these observations is, as yet, largely a matter of conjecture. It is possible that the beneficial action of calcium on the hemoglobin level may depend on the suggested ability of this element to exert a favorable effect on the economy of iron in its metabolism,[6, 7] a point under investigation at the present time. Also, the foregoing data are of importance because they show that the calcium-supplemented animals, although still consuming a ration extremely deficient in sodium and chlorine, and to a lesser degree potassium, magnesium and phosphorus, maintain a normal blood picture and, as far as can be determined by gross observations, are entirely normal.

[6] von Wendt, G., *Skand. Arch. Physiol.*, 1905, **17**, 211.

[7] Sherman, H. C., Bulletin 185, U. S. Dept. Agriculture, Office of Experiment Stations, 1907.

7983 P

Intermediate Oxidation Products of Epinephrine.

S. WEINSTEIN AND R. J. MANNING. (Introduced by J. Markowitz.)

From the Department of Chemistry, University of Saskatchewan.

Since Addison made clear the physiological importance of the suprarenal gland, a tremendous number of papers dealing with the physiopathological aspect of the subject have been published, but on the whole comparatively little work has been reported concerning the oxidation products of the active principle. Most chemical investigations have dealt with the oxidation of epinephrine to a pink color with the object of establishing a quantitative chemical assay. Although numerous methods have been reported, only the persulphate oxidation method gives comparable results with those obtained by biological assays. The present work was undertaken with the object of isolating if possible and identifying the chemical constitution of the red oxidation product or products of epinephrine.

Crystalline epinephrine was treated with anhydrous silver oxide in a manner described by Willstätter[1] for the oxidation of catechol to quinone. This procedure necessitated complete absence of water from the reacting substances. Crystalline epinephrine (0.5 gm.) was suspended in methyl alcohol. Three equivalents of silver oxide were added and the mixture shaken for 5 minutes. The mixture was filtered and, after reducing the volume of the filtrate in a vacuum desiccator, crystallization was effected in the cold by the addition of anhydrous ether. A fine red crystalline product separated which decomposed rapidly into a brown amorphous substance in a manner similar to the experience of Willstätter with orthoquinone. More stable crystals were obtained by allowing oxidation to take place in an inert medium such as amylene. The red product crystallized in bright red microscopic needles upon the silver oxide. It was impossible, however, to recover these crystals from various solvents or floating processes without decomposing them. The red product was removed from the silver oxide by dissolving it in methyl alcohol, and its constitution was identified as a mono methyl amino ethanol 3:4 quinone (epinephrine with the 2 hydroxyl groups on the benzene ring oxidized to keto groups) by the method described by Koch and Jackson[2] for the identification of quinone. Mono-

[1] Willstätter, R., *Ber. deutsch. Chem. Ges.*, 1904, **37**, 4744.

[2] Koch, W., and Jackson, C. L., *Ber. deutsch. Chem. Ges.*, 1898, **31**, 1457.

methylaminoethanol 3:4 quinone has no effect on blood pressure.

It was impossible to oxidize the secondary hydroxyl group in the side chain of the epinephrine molecule without disrupting the benzene ring. The epinephrine product with 3 keto groups (monomethylamino aceto 3:4 quinone) can be obtained however by starting with adrenalone and then oxidizing the catechol group to quinone. Adrenalone was prepared by a method employed by Dziergowski.[3] This product was dissolved in the minimum of 0.1 N HCl and aqueous ammonia added until yellow crystals of adrenalone began to appear. Ammonia was added further while the solution was vigorously stirred. The solution first turned pink, then red and finally a dark red crystalline product separated out upon standing in the cold. The crystals were washed with cold water and dried. The product had no effect on blood pressure. (Found: C, 60.4; H, 5.4; N, 8.0. $C_9H_9NO_3$ requires C, 60.3; H, 5.0; H, 7.8). Monomethylamino aceto 3:4 quinone is more stable than monomethylaminoethanol 3:4 quinone. The same characteristic is shown by epinephrine and adrenalone.

7984 P

Effects of Ingested Fats and Sterols on Sterol Metabolism of the White Rat.

H. C. ECKSTEIN.

From the Department of Physiological Chemistry, Medical School, University of Michigan, Ann Arbor.

In a study of the effects of the ingestion of plant fats and sterols on the metabolism of the white rat, young litter mates were placed on plant diets consisting of a mixture of soy bean meal, corn oil, agar, starch and the Osborne and Mendel salt mixture. The 2 diets used were so prepared that the protein intake would be practically the same for both groups of rats. The fat content of one diet was 11% as compared with 34% for the other. Both were supplemented with a vitamin B yeast concentrate (Harris), carotene, and viosterol. A record of food intake was kept. The sterol contents of the liver, the remaining tissues, the diets, and the feces were determined gravimetrically by means of the digitonin method. The table shows that

[3] Dziergowski, S. K., *Centralb.*, 1893, **2**, 861.

most of the rats on the high fat diet in series 1 and 3 had a higher percentage of liver sterols than those on the low fat diet. This is not true for series 2 in which not only poor growth was obtained but also in which the experimental period was increased by 3 weeks. The table also shows that the larger percentage of liver sterols cannot be due to sterol mobilization because of the similarity of the sterol contents of the rest of the tissues of all of the rats. Fats may therefore be the precursors of sterols. Such a view would be at variance with one held by Chanutin and Ludewig[1] who state that while carbohydrates cause a deposition of cholesterol in the white rat, fats inhibit that phenomenon. Further data showed that all of the rats synthesized sterols since the amounts excreted in the feces were always larger than those fed. In general this took place to a greater extent on the high fat diet. The source of the extra sterols has not been determined. According to Schönheimer[2] ergosterol and cholesterol are the only sterols absorbed, but Dam and Starrup[3] concluded that dietary "phytosterines" occasionally find their way to the livers of rats. The extra sterols may thus originate from ingested fat, from ingested sterols or from both.

TABLE I.
Sterol Contents of Livers and Remaining Tissues.

	Low fat-sterol diet			High fat-sterol diet		
	No. of rats	Liver sterols	Sterols in remaining tissues	No. of rats	Liver sterols	Sterols in remaining tissues
		%	%		%	%
Series 1 (7 wks.)	2	.41 (.40-.42)*	.19 (.19-.19)	2	.85 (1.00-.69)	.20 (.19-.20)
Series 2 (10 wks.)	3	.35 (.28-.42)	.19 (.18-.19)	3	.40 (.39-.41)	.20 (.20-.21)
Series 3 (7 wks.)	6	.39 (.30-.46)	.21 (.19-.23)	6	.61 (.43-.72)	.24 (.20-.29)

* The figures in parentheses represent the ranges of the values in the different experimental groups.

1 Chanutin, A., and Ludewig, S., *J. Biol. Chem.*, 1933, **102**, 57.

2 Schönheimer, R., *Z. physiol. Chem.*, 1929, **180**, 1.

3 Dam, H., and Starrup, U., *Biochem. Z.*, 1934, **274**, 117.

7985 C

Analysis of Type II Pneumococcus Specific Precipitate.

HERBERT O. CALVERY. (Introduced by H. B. Lewis.)

From the Department of Physiological Chemistry, Medical School, University of Michigan, Ann Arbor.

The antibodies of an immune animal are associated with the globulin fraction of the serum proteins. Since they are difficult to separate from the serum proteins in a relatively pure form, very few chemical investigations of them have been made. The most recent is that of Hewitt[1] in which are reported the amide-N, mono-amino-N, diamino-N, cystine, tyrosine, and tryptophane of diphtheria toxin-antitoxin floccules. These are compared with the crystalline albumin fraction and the globulin fraction from which the chemical methods used do not permit differentiation.

Careful chemical analysis of the precipitate formed when specific haptens are added to antisera should furnish valuable information since this precipitate contains antibody in the purest form in which it is available. The present investigation is of Type II pneumococcus specific precipitate furnished by Dr. M. Heidelberger and Dr. F. E. Kendall of Columbia University, to whom I wish to express my appreciation. The precipitate contains about 5% of the specific carbohydrate, and the analytical values have not been corrected for this. Analyses of other specific precipitates are in progress.

The specific precipitates were obtained, purified and dried in the usual manner of preparation of protein precipitates by filtration, washing with water, alcohol, and ether and subsequently placing in a vacuum desiccator over some drying agent. Two samples of the white powder thus obtained were analyzed by the general chemical methods of protein analysis in use in this laboratory.[2] The analytical values obtained are summarized in Table I. All values reported are averages of duplicate analyses for the 2 samples except the isolation values obtained for arginine, histidine, lysine, glutamic acid and aspartic acid.

The values found agree in a general way with those reported for serum globulin but before specific comparisons can be made more careful analyses must be carried out on highly purified fractions of the serum globulins, if possible from the same animals from which the specific precipitates have been obtained. The cystine

1 Hewitt, L. F., *Biochem. J.*, 1934, **28**, 2080.

2 Calvery, H. O., and Freyberg, R. H. To appear in *J. Biol. Chem.*, 1935, **109**.

TABLE I.
Analytical Values for Type II Pneumococcus Specific Precipitate.
All values are corrected for ash and moisture. The nitrogen fractions are expressed as percentages of the total nitrogen.

	I %	II %
Ash	0.16	0.18
Moisture	6.0	6.1
Total N	15.9	16.0
Amide N	3.7	3.6
Humin N	0.58	0.66
Amino N (after hydrolysis)	76.8	73.4
Phosphorus	none	none
Sulfur	1.3	1.2
Tyrosine	5.5	5.5
Tryptophane	2.0	2.0
Cystine	3.1	3.1
Arginine	5.7	5.4
Histidine	1.0	1.1
Lysine	4.9	4.7
Aspartic Acid	4.3	4.5
Glutamic Acid	6.1	6.4

values in Table I are much higher, the tryptophane values slightly higher and the tyrosine values somewhat lower than those reported by Hewitt[1] for diphtheria toxin-antitoxin floccules, for which amino acids the mean values are 2.05%, 1.80% and 5.85% respectively. The amide N value found by Hewitt is very high, being 9.1% as compared to 3.65% in the table. From these comparative values it seems very probable that more complete chemical analyses of highly purified products will afford valuable information concerning the chemical composition of antibodies, their probable method of formation and the nature of the reactions between antigens and antibodies.

7986 C

Specific Rotation of Cystine Excreted in Cystinuria.

BARKER H. BROWN AND HOWARD B. LEWIS.

From the Department of Physiological Chemistry, Medical School, University of Michigan, Ann Arbor.

The identity of stone cystine and protein cystine has been generally accepted in recent years. Gortner and Hoffman[1] in an examination of cystine isolated from kidney calculi observed a specific ro-

[1] Gortner, R. A., and Hoffman, W. F., PROC. SOC. EXP. BIOL. AND MED., 1926, **23**, 691.

tation at 20° in 0.1 N hydrochloric acid of —242.6°, a figure much higher than any value recorded in the literature for either stone or protein cystine. They believe that the conflicting observations make necessary the conclusion "that cystine is an extremely labile compound and possibly occurs in more than one form so that persons working with cystine are probably working with a mixture of substances and that this mixture varies in composition depending at least upon (1) the source of the biological material from which the cystine is prepared, and (2) the method of preparation which is used for the isolation and purification of the amino acid." The different values for specific rotations of cystine recorded in the literature can in all probability be explained by varying degrees of racemization or by failure to use standard conditions for the determination of the specific rotation,[2, 3] but it remains to be demonstrated that the optical properties of cystine vary with the biological source of the amino acid.

In studies with a cystinuric patient we observed that crystals, which were observed to be almost pure cystine on microscopic examination,[4] separated very rapidly after the sample was collected. Since the pH of the urine was 6.8 to 7.2, an opportunity was afforded to determine the specific rotation of the cystine excreted in cystinuria, a cystine which had not been subjected to high concentrations of acid or alkali and which should show minimal racemization.

The urine was filtered through a Buchner funnel with fritted glass disc; the precipitate was repeatedly washed with distilled water and with a small volume of 10% acetic acid to remove any phosphates. After further washing with water, the cystine was dissolved on the filter in a small volume of 3% hydrochloric acid. The filtrate was immediately treated with a saturated solution of sodium acetate and the precipitated cystine was washed repeatedly by centrifugation with distilled water and finally with alcohol and with ether. The cystine was once recrystallized under the same conditions and the product was dried for 6 hours in an oven at 95-100° and then in a desiccator over phosphorus pentoxide for 2 months. The cystine thus obtained had not been in contact with alkali or acid for any considerable period of time.

This cystine was compared with a sample of cystine from protein

2 Andrews, J. H., *J. Biol. Chem.*, 1925, **65**, 147.

3 Toennies, G., and Lavine, T. F., *J. Biol. Chem.*, 1930, **89**, 153.

4 A microphotograph of typical urinary cystine crystals of this patient is shown in Lewis, H. B., *Ann. Internal Med.*, 1932, **6**, 183.

showing maximal optical activity[2] generously placed at our disposal by Professor J. H. Andrews of the University of Pennsylvania. When analyzed by the Sullivan-Lugg method, the urinary cystine showed a purity of 99.7% as compared with a purity of 99.5% for the protein cystine, values within the range of accuracy of the Sullivan-Lugg procedure.[5]

0.5 gm. samples of each of these cystines were dissolved in 50 cc. of N hydrochloric acid and the rotations determined in 2 dcm. tubes at 30° using sodium light. With the urinary cystine an $[\alpha]_D^{30°}$ of —201.0° was obtained; with the protein cystine, a rotation under the same conditions of —202.5°. As a further check on our determinations, the optical activities of the 2 cystines were determined independently by Professor Andrews. Values of —214.0° and —215.0° at 25° respectively were obtained.

If our values are corrected to a temperature of 20°, using the factor of Toennies and Lavine,[3] the specific rotations are —221.6° and —223.1° respectively, values which compare favorably with the classical values of Fischer and Suzuki,[6] —223.6° for stone cystine and —221.9° for cystine from hair.

These data offer no evidence that cystine which crystallized spontaneously from cystinuric urine and which had had minimal opportunity for racemization, differed significantly in its specific rotation from the maximal values usually given for the rotation of *l*-cystine from protein hydrolysates.

7987 P

Charcoal Adsorption as a Method for the Preparation of a Concentrated Liver Extract.

JEAN L. KYER. (Introduced by Raphael Isaacs and Cyrus C. Sturgis.)

From the Thomas Henry Simpson Memorial Institute for Medical Research, University of Michigan, Ann Arbor.

It has been noted that in concentrating liver extracts for the treatment of pernicious anemia to very small volumes a considerable degree of potency was lost in the precipitate which formed. In the search for a satisfactory method of obtaining a concentrated liver extract which could be administered intramuscularly and which

[5] Lugg, J. W. H., *Biochem. J.*, 1933, **27**, 668.

[6] Fischer, E., and Suzuki, V., *Z. physiol. Chem.*, 1905, **45**, 405.

was as potent as a dilute preparation derived from 100 gm. of liver, the following procedure was developed.

Fresh liver is ground and suspended in water in the proportion of 5 lb. of liver to 5 liters of water. This suspension is stirred for 15 minutes and then heated to 80°C. in a water bath. The solution is filtered off, and the residue is washed with hot water and filtered again. The combined filtrates are concentrated to about one-fourth of their volume and a 25% neutral lead acetate solution is added until precipitation no longer occurs. The precipitate is filtered off and enough baryta-water is added to the filtrate to give complete precipitation. The precipitate is filtered off and sulfuric acid is added to the filtrate to precipitate the lead and the barium. This suspension is centrifuged, and the clear yellow filtrate is poured off and brought to pH 5.0 with sodium hydroxide. Norite charcoal (about 25 gm. for every initial 5 lb. of liver) is then added to the solution and the mixture is allowed to stand for an hour with frequent shaking. The charcoal is filtered off, washed, suspended in 50% alcohol (400 cc. per 25 gm. charcoal) adjusted to pH 5.0, and heated to 65-70°C. on a steam bath. The alcoholic solution is removed and the precipitate is again treated with 50% alcohol. The combined alcoholic filtrates are concentrated under reduced pressure and reduced in volume until 3 cc. of the solution represent 100 gm. of fresh liver. This solution is then filtered, boiled in a water bath for a few minutes, cooled in the ice box over night, filtered again, bottled, and sterilized. Final pH = 5.

The clear yellow extract was given intramuscularly in a 3 cc. dose to a patient with pernicious anemia whose red blood cell count was 0.80 millions per cu. mm. before transfusion and 1.43 millions per cu. mm. after transfusion the day treatment began. The reticulocyte peak was 52.4% in 5 days, and in 7 days the red blood cell count was 1.94 millions per cu. mm. and the hemoglobin was 35%. After 3 more injections at weekly intervals the red blood cell count was 3.91 millions per cu. mm. and the hemoglobin was 74%.

Further studies are now being made on the properties of this concentrated extract, and it is being tested clinically for immediate response to treatment and also for maintenance of a normal red blood cell count.

Summary. A method for the preparation of liver extract based on the property of the hematopoietically active principle of becoming adsorbed by charcoal from an acid solution is described. This allows the concentration of the fluid to a small volume without much loss of potency.

7988 C

Equivalence-Point Ratio of Antibody to Antigen in Ovalbumin Precipitates.*

SANFORD B. HOOKER AND WILLIAM C. BOYD.

From Evans Memorial (Massachusetts Memorial Hospitals) and Boston University School of Medicine.

Heidelberger and Kendall[1] first showed that the variable composition of antibody-antigen (pneumococcus III) precipitates depends upon the proportions of antiserum and antigen, and that the precipitate formed at the equivalence-point has an approximately constant composition relatively independent of the absolute concentrations of the reagents or of the potency of any particular lot of serum.

The equivalence-point ratio varies with different antigens but its systemic constancy affords an obviously important reference point for future quantitative study of certain immune reactions. One of the best studied antigens is crystalline ovalbumin, and it should be helpful to establish limits of error within which we can determine the equivalence-point ratio. Three previous determinations[2, 3, 4] varied significantly.

The equivalence-point is indicated by that mixture (ratio) of serum and antigen in whose supernatant there remains neither antibody nor antigen or but minimal traces of both, after precipitation is complete. For several systems, including ovalbumin and its antibody, this is identical with the constant antibody optimum.[5]

Our hen-ovalbumin was recrystallized 5 times and evidence of its very high purity has been presented.[6] Precipitates formed in neat serum at the Dean and Webb optimum (37°C., 30′) were allowed to stand in the ice box over night, centrifuged, and with thorough dispersion washed 3 times with 1 ml. chilled saline. The precipitates were dissolved in weak NaOH and digested with 1 ml. H_2SO_4. Nitrogen was determined by the micro-Kjeldahl method.[7] The *clear* stock ovalbumin solutions were similarly standardized, tested for

*Aided by a grant from the National Research Council.

1 Heidelberger, M., and Kendall, F. E., *J. Exp. Med.*, 1929, **50**, 809.

2 Culbertson, J. T., *J. Immunol.*, 1932, **23**, 439.

3 Taylor, G. L., Adair, G. S., and Adair, M. E., *J. Hyg.*, 1934, **34**, 118.

4 Heidelberger, M., and Kendall, F. E., *J. Exp. Med.*, 1934, **59**, 519.

5 Dean, H. R., and Webb, R. A., *J. Path. and Bact.*, 1926, **29**, 473.

6 Hooker, S. B., and Boyd, W. C., in press.

7 Parnas, J. K., and Wagner, R., *Biochem. Z.*, 1921, **125**, 253; see Pregl, F., *Die Quantitative Organische Mikroanalyse*, 3rd ed., J. Springer, Berlin, 1930.

residual ammonia and any necessary correction applied. The supernatants from the precipitates were tested serologically for antigen and for antibody; *neither was ever found.*

Typical quadruplicate results obtained with precipitin 717-8-9, a pool of 3 different rabbit-sera, are detailed in Table I together with the ratios observed with other antisera. A summary of our means and those of previous investigators is given in Table II.

TABLE I.
Nitrogen (mg.) in Ovalbumin Anti-ovalbumin Precipitates.

Serum	N in ppt.	Antigen N	Difference (antibody N)	Ratio* ab/an
717-8-9	0.764	0.069	0.695	10.08
"	.750	.069	.681	9.86
"	.764	.069	.695	10.08
"	.754	.069	.685	9.93

Serum 671-2-3 (also a pool of 3) gave ratios 9.40, 9.55, 9.73, 10.02; serum 610, 10.46, 9.73, 9.77, 9.95.

* This ratio is actually antibody-N/antigen-N.

The mean of our individual ratios (Table II) is 9.99, with a σ_M (standard deviation of the mean) of ± 0.10.

TABLE II.
Means of Determinations of Antibody-antigen Ratios for Different Sera. Means of Results of Other Investigators.

Serum	Antigen solution	Mean	σ_M	Author	Mean	σ_M
610	1	9.98	.17	Culbertson	13.06	.34
671-2-3	"	9.67	.13	Taylor, Adair and Adair	10.15	.25
717-8-9	"	9.99	.06	Heidelberger and Kendall	11	—
671-2-3	2	9.82	.55	Hooker and Boyd	9.99	.10
717-8-9	"	10.47	.09			
All		9.99	.10			

Two other sera 621-2, from rabbits injected with ovalbumin coupled with arsanilic and iodosulfanilic acids, contained no antihaptens for these prosthetic groups but did precipitate ovalbumin strongly. Equivalence-point ratios obtained with these pooled sera were 8.59, 8.76, 8.91, 8.62, σ_M .07. These figures further indicate the consistency of the analytic method but when we apply Fisher's[8] test of "t" it becomes apparent that their mean differs significantly from the consistent means of 610, 671-2-3, and 717-8-9 (t about 6, $n = 6$) with the same lot of antigen (Table II). Inasmuch as sera

[8] Fisher, R. A., *Statistical Methods for Research Workers,* 4th ed., Oliver and Boyd, Edinburgh, 1932.

621-2 reflect a *modified* ovalbumin, some of whose native antigenic determinants were fundamentally altered by conjugation with the diazonium compounds, it is reasonable to suppose that their antibody would differ qualitatively from "natural" antiovalbumin, would find fewer locations on the surface of ovalbumin molecules with which it could combine,[4, 6] and so a lower ab/an ratio would result. In support of this assumption we found that serum 621-2 failed to precipitate *duck*-ovalbumin—a result we have never observed with numerous antisera to natural hen-ovalbumin.[6]

The "t" test does not distinguish our mean from that of Taylor, Adair and Adair, but Culbertson's differs significantly. In the absence of Heidelberger and Kendall's original data or standard deviation—their citation of their ratio was incidental—we can not be sure that our mean also differs significantly from theirs; probably it does. We have, therefore, combined our results and those of Taylor, *et al.*, and obtain a mean of 10.05 with a σ_M of 0.13. We are inclined to think this approaches closely to the true value of the ratio but the higher results of Culbertson and of Heidelberger and Kendall remain to be explained. Perhaps a constant error affects either our results and those of Taylor, *et al.*, or those of Culbertson and perhaps Heidelberger and Kendall. An error in standardizing the antigen solution could be considered. In any case our figures serve to give an idea of the inherent variation to be expected in determinations of this sort.

Possibly the ratio may be slightly altered when the N content of rabbit-antibody is actually determined. The percentage of N in horse-serum-globulin, and thus, probably, in antibody, differs little from that in ovalbumin (about 15.6). Felton's[9] figures for highly purified equine antipneumococcus precipitin range from 14.9 to 15.7. Taylor, *et al.*, found the percentage of N in some of their precipitates, consisting of 90% rabbit-antibody, to be also about 15.2. Thus the correction could not be great.

Summary. Another determination of the equivalence-point ratio of antibody to antigen in precipitates formed by crystalline ovalbumin and its antisera statistically conforms with one of the previously reported determinations, but differs significantly from the 2 others. The 2 sets of consistent determinations give a mean of 10.05, σ_M 0.13.

[9] Felton, L. D., *J. Immunol.*, 1932, **22**, 453.

Pacific Coast Section

Mount Zion Hospital, San Francisco, March 20, 1935.

7989 P

Effect of Pregnant Mare's Serum on the Immature Fowl.

V. S. ASMUNDSON AND M. J. WOLFE. (Introduced by H. H. Cole.)

From the College of Agriculture, University of California, Davis.

Precocious development of immature male fowl has been induced by injection of extracts from the anterior lobe of the pituitary[1, 2] and by pregnant mare's serum.[3] Less marked response of the ovary, oviduct and head furnishings to extracts of the anterior pituitary has been obtained in the immature female fowl[1] while oestrin induces precocious development of the oviduct only.[4] In the immature pigeon both the testes and ovary responded to injections of pregnant mare's serum, the increase in the weight of the ovary being less than that of the testes.[5] The effect of pregnant mare's serum on the immature female fowl has not been reported.

For the experiments here reported pregnant mare's serum containing 50 rat units per cc. (obtained from Dr. H. H. Cole) and oestrin containing 1,000 rat units per cc. prepared by the method of Leonard, Hisaw and Fevold[6] from pregnant mare's urine, were used. The birds were injected daily for the duration of the experimental periods.

In a series of experiments immature white Leghorn males were injected with pregnant mare's serum. The results confirm those of Hamburger[3] for short periods of injection, the size of the testes

1 Domm, L. V., and Van Dyke, H. B., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 349, 351.

2 Shockaert, J. A., *Am. J. Physiol.*, 1933, **105**, 497.

3 Hamburger, Chr., *Endokrinol.*, 1934, **13**, 305.

4 Juhn, M., and Gustavson, R. G., *J. Exp. Zool.*, 1930, **56**, 31.

5 Evans, H. M., and Simpson, M. E., *Anat. Rec.*, 1934, **60**, 405.

6 Leonard, S. L., Hisaw, F. L., and Fevold, H. L., *J. Am. Chem. Soc.*, 1932, **14**, 254.

being increased as much as 6 times, and the comb being much enlarged. In a preliminary experiment 2 birds injected with 4 cc. (200 rat units) of mare's serum daily from 42 to 91 days of age were found to have smaller testes than the control. One of these mated repeatedly with bantam hens but no fertile eggs were obtained. This agrees with histological examination of the testes since, although the tubules and the interstitial tissue were much enlarged, in no case were spermatozoa present. Short periods of injection of pregnant mare's serum have also been found to increase the size of the testes of immature 128-day-old Bronze turkey males (left testis; injected 1411 mg.; control 198 mg.).

The results with immature white Leghorn females are shown in Table I. In each case the weight of the ovary increased as a result of injecting the pregnant mare's serum but no ripe follicles were produced even after prolonged injection. The oviduct was much enlarged in every case. Injection of oestrin, if it had any effect, reduced slightly the weight of the ovary but increased the size of the oviduct. Where both pregnant mare's serum and oestrin were injected, the oviduct was larger than when oestrin alone was injected. The vagina ended blindly in all of these birds except Nos. 24 and 25. The vagina does not ordinarily open in Leghorns until the birds are somewhat older than this or at about 135 to 150 days according to Palmer.[7]

TABLE I.
Effect of Pregnant Mare's Serum and Oestrin on Immature Female Fowl.

Bird No.	Age killed in days	Wt. when killed gm.	Amt. injected daily: Pregnant mare's serum rat units	Amt. injected daily: Oestrin rat units	Injection Days	Wt. ovary mg.	Length oviduct mm.	Size comb mm.
1	63	408	0	0	—	198	80	small
2	63	568	150	0	42	509	360	70x32
11	117	1243	0	0	—	280	105	32x15
12	117	1120	250	0	10	448	300	60x31
20	99	1025	0	0	—	275	100	35x19
21	99	950	0	1000	10	243	270	38x14
22	99	965	250	1000	10	536	400	45x25
23	115	1164	0	0	—	452	90	35x20
24	115	1280	0	1000	26	425	340	35x15
25	115	1188	250	1000	26	643	490	43x23

The injection of oestrin had no effect on the comb. On the other hand, pregnant mare's serum increased the size of the comb in every case. The comb did not, however, become turgid and erect like that of injected immature males but tended to lop over like that of nor-

[7] Palmer, V. E., unpublished data, Univ. of B. C., 1932.

mal Leghorn females. The effect on the comb is, therefore, apparently different from the effect of injecting Hebin which induces the development of a comb similar to that of the male.[8] It is also of interest to note that while No. 1 had the pale earlobe characteristic of birds of this age and breed, No. 2 had the large, enamel white earlobe characteristic of mature Leghorn females.

The results of these experiments show that pregnant mare's serum induces precocious sexual development up to a certain point. There is a marked increase in the size of the testes and a smaller, but definite increase in the size of the ovary. While the evidence is not complete there is some indication that neither spermatogenesis nor ovulation can be induced in immature birds by the injection of pregnant mare's serum. The oviduct in the female is much enlarged and the head furnishings approximate those of mature birds but are typical of the sex of the birds, whether male or female.

7990 P

Effects of Tartar Emetic on the Leukocyte Count.

S. P. LUCIA. (Introduced by D. M. Greenberg.)

From the Division of Medicine, University of California Medical School, San Francisco, California.

When tartar emetic is inoculated into rabbits it produces a leukopenia, without secondary leukocytosis, and without affecting the erythrocyte count.[1, 2] Because of this property it was considered advisable to study the possible action of this drug on various types of leukocytoses occurring in man. For this purpose a freshly prepared 1% solution of potassium antimonyl tartrate in sterile distilled water was administered intravenously, in progressively graded doses of 2, 3 and 5 cc. on alternate days.

Antimony is irritating when inoculated directly into tissue. When given intravenously, care must be exercised not to allow any of it to seep out of the veins. In certain cases it was noted that the patients complained of slight nausea during the inoculation; others experienced a sensation of warmth associated with blushing, particularly of the face.

---

8 Domm, L. V., *Anat. Rec.*, 1934, **60**, supplement p. 50.

1 Lucia, S. P., and Brown, J. W., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 426.

2 Lucia, S. P., and Brown, J. W., *J. Pharm. and Exp. Therap.*, 1934, **52**, 418.

Case I. Mr. R., aet 36. Chronic lymphatic leukemia. W.B.C. 300,000 per cu. mm. Five inoculations of 2, 3, 3, 5 and 5 cc. respectively were given over a period of 13 days. The leukocyte count after the course of treatment dropped to 75,000 per cu. mm. Lymphadenopathy and splenomegaly were not affected.

Case II. Mrs. T., aet 70. Hodgkin's disease. W.B.C. 25,950 per cu. mm. Three inoculations of 3 cc. were given on successive days. The leukocyte count following treatment was 15,950 per cu. mm. Shortly after each inoculation there was an abrupt drop in the cell count, followed by a gradual rise.

Case III. Mrs. C., aet 40. Eosinophilic leukocytosis. W.B.C. 34,650 per cu. mm., with 71% eosinophiles. Four inoculations of 3, 5, 5 and 5 cc. respectively were given over a period of 8 days. The leukocyte count following therapy was 10,800 per cu. mm., with 22% eosinophiles.

Case IV. Mr. C., aet 72. Chronic lymphatic leukemia. W.B.C. 150,000 per cu. mm. Five inoculations of 3, 5, 5, 5 and 5 cc. respectively were given over a period of 10 days. After the fourth inoculation the leukocyte count dropped to 75,000 per cu. mm. Lymphadenopathy and splenomegaly were not affected. At this point the course was complicated by a sterile abscess at the site of inoculation, followed by a local herpes. The leukocyte count rose to 120,000, and after the fifth inoculation antimony treatment was discontinued. Later the administration of Fowler's solution was likewise without effect.

Case V. Miss C., aet 36. Chronic myelogenous leukemia. W.B.C. 137,000 per cu. mm. Six inoculations of 3, 3, 5, 5, 5 and 5 cc. respectively were given over a period of 19 days. The leukocyte count following the course of therapy was 4,500 per cc. The spleen further increased in size and became tender after treatment. This patient died 5 weeks after the last inoculation.

Case VI. Miss A., aet 32. Chronic myelogenous leukemia. W.B.C. 40,000 per cu. mm., with 26% immature forms. Four inoculations of 2, 3, 5 and 5 cc. respectively were given over a period of 9 days. The leukocyte count after the course of therapy was 12,000 per cu. mm., with 7% immature forms.

Case VII. Mrs. M., aet 38. Chronic myelogenous leukemia. W.B.C. 257,000 per cu. mm. Seven inoculations of 3, 5, 5, 5, 5, 5 and 5 cc. respectively were given over a period of 16 days. The leukocyte count following the course of therapy was 202,000 per cu. mm., and the spleen was reduced 7 cm. in length and in breadth.

Case VIII. Mrs. S., aet 52. Advanced monocytic leukemia,

with massive skin infiltrations. This patient had failed to react to Fowler's solution. W.B.C. 96,000 per cu. mm. Five inoculations of 3, 5, 5, 5 and 6 cc. respectively were given over a period of 12 days. The leukocyte count after therapy was 192,000 per cu. mm. The patient died a few days after the last inoculation.

Case IX. Mr. C., aet 46. Chronic myeloid leukemia. W.B.C. 162,000 per cu. mm. Eleven inoculations of 5 cc. each were given over a period of 20 days. The leukocyte count following treatment was 47,000 per cu. mm. During the treatment the spleen decreased in size. The patient was then given Fowler's solution and the leukocyte count gradually increased. After it had reached 346,000 per cu. mm., 5 inoculations of tartar emetic were given and the count dropped to 210,000. This patient died 3 months later.

Conclusion. Following intravenous inoculation of one per cent solution of potassium antimonyl tartrate, the leukocyte count was reduced in 6 of 9 patients who exhibited leukocytoses of abnormal cells.

7991 P

Nature of Formalin Inactivation of Bacteriophage.

E. W. SCHULTZ AND L. P. GEBHARDT.

From the Department of Bacteriology and Experimental Pathology, Stanford University, California.

Immunologists and students of the filtrable viruses are familiar with the fact that formalin possesses the unique property of converting toxins to toxoids and viruses to what may be called "virusoids" and of doing this without greatly impairing the antigenicity of these agents. While various physical and chemical agents readily inactivate toxins and viruses, such inactivation is generally associated with complete loss of antibody stimulating powers. The unique property which formalin possesses has interested us for some time. Schultz, Quigley and Bullock,[1] in discussing the antigenicity of formalin inactivated bacteriophage suspensions stated that "it is by no means clear that formalin actually kills or effaces the identity of the virus." We have felt for some time that the preservation of the antigenicity of formalin inactivated toxins, bacteriophage and of

[1] Schultz, E. W., Quigley, J. S., and Bullock, L. T., *J. Immunol.*, 1929, **17**, 245.

animal viruses might possibly rest on some common mode of action—one in which the toxin or virus is not permanently altered, but is held in an inactive state only so long as the formalin is combined with the antigen, and that the antigenicity of formalin inactivated toxins or viruses might be explained on the basis of a "dissociation" which is later effected within the body. It seems possible that the inactivation itself may be of the nature of an ordinary "formol reaction" in which formaldehyde combines with the amino group to form a methylene derivative and that after injection into the body the formalin radical in the new complex may be removed by some chemical process, probably oxidative in character, which restores the toxin or virus to its native state. The difficulty in testing such an hypothesis consists, of course, in finding a method which will release or destroy the linked formaldehyde without at the same time destroying the toxin or virus.

We have recently been successful in reactivating formalin inactivated staphylococcus bacteriophage suspensions by a very simple procedure. A certain staphylococcus bacteriophage suspension (Ph. 127—A.D. 100), which is normally active in a dilution of 10^{-9}, is completely inactivated by 0.018% formaldehyde (HCHO) in 24 hours at 37°C. If one adds from 1 to 5 cc. of such an inactivated suspension to 100 cc. or more of distilled water (pH 6 to 6.6) and stores this at 37°C., active phage is released. The reactivation proceeds slowly and does not approach completion until after a period of 10 to 15 days.

Studies are now in progress to determine whether reactivation of other viruses and of toxins can be accomplished by appropriate methods, including the use of mild oxidizing agents. We have, however, proceeded far enough in these studies to say that the formalin inactivated poliomyelitis virus cannot be reactivated by the simple procedure which suffices to reactivate bacteriophage.

7992 P

What Is Cyanmethemoglobin?

MATILDA MOLDENHAUER BROOKS

From the University of California, Berkeley.

Since previous workers[1] have explained the action of nitrites and methylene blue in cyanide poisoning as the formation of methemoglobin by these substances and the consequent union of CN with the methemoglobin to form cyanmethemoglobin, I was interested in finding out what constituted cyanmethemoglobin.

The literature is rather vague concerning this substance. The few brief descriptions are not quantitative and there seems to be a dispute as to its chemical configuration.[2]

I therefore studied the effects of either or both KCN and $NaNO_2$ on the spectrophotometric picture of oxyhemoglobin and reduced hemoglobin. The amount of $NaNO_2$ employed was sufficient to change 88% of the oxyhemoglobin to methemoglobin, and the chemically equivalent amount of KCN was used. The resulting hemoglobin derivatives were examined spectrophotometrically.

Oxyhemoglobin was formed by vigorous shaking either in air or oxygen; reduced hemoglobin by means of Stokes Reagent according to the usual procedure; methemoglobin, by adding $NaNO_2$. *In vitro* experiments with sheep, rat and rabbit blood and *in vivo* experiments with rabbit and rat gave essentially similar results. The experiments here reported were done with sheep red blood cells *in vitro*. To each 2 cc. of defibrinated blood which had been washed several times and made up to original volume in 0.9% NaCl was added 0.2 cc. of 0.33 M $NaNO_2$ and/or KCN according to the experiment. For spectrophotometric examination 1 cc. of the resulting suspension was hemolyzed in 100 cc. of 0.4% NH_4OH.

In no case did the absorption spectrum in the presence of KCN differ qualitatively from that of oxyhemoglobin. The ratio of the extinction coefficients at 540 and 560 mμ was calculated and is given as R in Table I. This ratio indicates according to Ray, Blair and Thomas[3] the proportion of oxyhemoglobin in mixtures with reduced or methemoglobin.

---

1 Wendel, W. B., *J. Am. Med. Assoc.*, 1933, **100**, 1054; Hug, E., *Compt. Rend. Soc. Biol.*, 1933, **112**, 511.

2 Hammarsten, O., *A Textbook of Physiological Chemistry*, 7th edition, 1914; Stadie, W. C., *J. Biol. Chem.*, 1920, **41**, 237.

3 Ray, G. B., Blair, H. A., and Thomas, C. I., *J. Biol. Chem.*, 1932, **98**, 63.

TABLE I.

Form of hemoglobin	Value of R	% oxyhb.
Oxyhb. alone	1.63	100
Oxyhb. + KCN	1.62	
Methb. alone	1.28	12
Methb. + KCN	1.63	
Reduced Hb. alone	0.849	0
Reduced Hb. + KCN	1.62	

Table I shows that in the case of oxyhemoglobin alone the appropriate value of R was obtained. An identical absorption curve and value of R was obtained for oxyhemoglobin plus KCN. The methemoglobin preparation was found to contain 12% oxyhemoglobin (the remainder being methemoglobin), but when KCN was added to this, the absorption curve and R value immediately shifted to that characteristic of a pure oxyhemoglobin preparation. Similar results were obtained when KCN was added to reduced Hb. These readings were the same 2 hours later but 48 hours later the solutions containing $NaNO_2$ gave the methemoglobin curve. These experiments were done at room temperature, but even when the blood cell suspensions containing KCN were warmed to 40°C. and were allowed to stand 10 minutes, the oxyhb. spectrum persisted.

It is tempting to conclude that since the cyanide containing suspensions gave the typical oxyhb. spectra, therefore the effect of the cyanide was to change all the hemoglobin to oxyhemoglobin and stabilize it. However, since the colors of the hemoglobin derivatives depend upon the configuration of the electrons around the Fe of the heme, it is conceivable that electron configurations could be set up which would be enough like those of oxyhemoglobin to give a spectrum indistinguishable from that of oxyhemoglobin. The observation of Cook[4] who states that when cyanide is added to oxyhemoglobin no O_2 is evolved, is interesting in this connection. This may mean that the O_2 is taken up by some other part of the molecule and therefore not released for measurement.

In conclusion it may be stated that no evidence for the existence of cyanmethemoglobin was found; that the absorption curve for cyanhemoglobin was identical with that for oxyhb., and that this was found to be the case regardless of whether the cyanide was added to oxyhemoglobin, or reduced hemoglobin, or methemoglobin.

[4] Cook, S. F., *J. Gen. Physiol.*, 1928, **11**, 339.

7993 C

Inability of Testicular Hormone to Masculinize Plumage and Eye-Color of Female Brewer's Blackbird.*

C. H. DANFORTH AND JEROME K. FISHER.

From the Department of Anatomy, Stanford University.

Attention has recently been called to a relation between testicular hormones and some secondary sexual or "ambosexual" characters in several avian species, but it is still too early to say how widespread the observed reactions may be. In the English sparrow, Keck[1] has found the color of the beak an excellent indicator of the amount of testicular hormone circulating in the blood, while the plumage is not such an indicator; van Ooordt and Junge[2] find that in one of the gulls a testicular hormone is necessary for development in the male of colors in bill and feet which are found in both sexes during the breeding season; and Gallagher, Domm and Koch[3] report that injection of purified testicular extract restores the plumage of Sebright capons to its normal form and color. In light of these findings, and since plumage and eye-color of the male blackbird (Euphagus cyanocephalus) seem to be unaffected by the injection of theelin,[4] it was thought desirable to see if secondary sexual characters in the females of this species would respond to the male hormone.

The substance used for the purpose was an extract prepared from beef testes by the procedure of Gallagher and Koch.[5] Following the final acetone extraction, the material was in each case evaporated to a thick paste and injected directly or first taken up in olive oil. It proved to be somewhat irritating to blackbirds but was readily tolerated by capons on which it was tested and in which it produced the expected effects on comb and plumage.

Three tests were made on adult females weighing about 60 gm. each: one in July, just after the breeding season; one in August; and one in March. Untreated males and females were kept as con-

* Supported in part by a grant from the Rockefeller Fluid Research Fund to the Stanford University School of Medicine.

1 Keck, Warren N., *J. Exp. Zool.*, 1934, **67**, 315.

2 van Ooordt, G. J., and Junge, G. C. A., *Acta brevia, Neerl.*, 1933, **3**.

3 Gallagher, T. F., Domm, L. V., and Keck, Fred C., *J. Biol. Chem.*, 1933, **100**, xlvii.

4 Danforth, C. H., and Price, John, Proc. Soc. Exp. Biol. and Med., 1935, **32**, 675.

5 Gallagher, T. F., and Koch, Fred C., *J. Biol. Chem.*, 1929, **84**, 495.

trols. The former are prevailingly blue-black with nearly white irises, the latter rusty black with dark brown irises. Injection into the pectoral muscles of the test females was begun from 6 to 10 days after dull feathers had been plucked from regions of the head and neck which are iridescent black in the male. The preparation employed for the first 2 tests was assayed by the capon method before and after these tests. From June 27 to June 30 a capon weighing about 1660 gm. was given 1.2 cc. of undiluted extract in 4 daily doses of .3 cc. each. By July 3 his comb had increased in length from 47.5 mm. to 57 mm., and in height from 20 mm. to 31 mm., after which it slowly regressed. From August 21 to August 25 this capon received a total of 1.1 cc. of the same extract, following which measurements of the comb showed an average increase of 24%, indicating no great loss of potency during this time. A female blackbird was given .15 cc. of the extract on July 12, and from .15 to .30 cc. on July 13, 14, 17, 19, 20, 21, 28, 31 and August 2. The total was 2.35 cc. No effect on eye-color or plumage was detected. A second series of injections was begun on August 20, at which time all the birds of both sexes were moulting. A total of 1.5 cc. of extract was administered in 5 injections given twice daily. The bird did not survive this treatment but at the time of death showed no change in eye-color (or plumage).

In March a new extract diluted in oil was used, of such potency that 12 cc. in 3 injections during 50 hours gave a 26% increase in comb measurements of a 1780 gm. capon and 8 cc. in 2 injections during 21 hours gave a marked effect on developing feathers of a 900 gm. Sebright type capon. This extract was administered to 3 female blackbirds, only one of which survived in good condition till final conclusions could be drawn. This bird received 1 cc. on the first day, 2 cc. (in separate doses) on the second, 2 cc. on the third and .5 cc. on the fourth, a total of 5.5 cc. No effect of this treatment on plumage or eye-color was detected.

In the first of these tests the amount of extract administered in 3 different sequences, *viz.*, July 12-14, July 19-21 and probably July 28-August 2, was in each case at least half as much as would have been necessary to produce an effect on a capon about 27 times as heavy, and on a few occasions during this period single doses were absolutely as great as those which produced an effect on such a capon. In the third test the dosage was at least 10 or 12 times as great in proportion to weight as that which had produced a marked influence on the tested capons. Failure to get results with the blackbirds is probably not due to inadequate dosage. It is possible, how-

ever, that some factor, such as inability to absorb this particular extract, is responsible for the negative findings, but it seems more probable that sexual differences in plumage and eye-color of this species have been differentiated with reference to factors other than the primary sex hormones.

7994 C

Antifibrinolytic Titer of Commercial Antistreptococcus Serums.*

J. K. VAN DEVENTER. (Introduced by W. H. Manwaring.)

From the Laboratory of Bacteriology and Experimental Pathology, Stanford University, California.

Twenty-eight commercial antistreptococcus serums† have been titrated for their neutralizing power (a) against the specific anti-human streptococcus fibrinolysin of Tillett and Garner,[1] and (b) against the two antiveterinary streptofibrinolysins of Madison.[2] The highest serial dilution of each antiserum completely neutralizing an arbitrary fibrinolytic dose of the selected streptococcus filtrates was taken as its approximate titer. Data thus obtained are recorded in Table I.

The table shows that each of 3 animal species tested is relatively immune to the homologous streptofibrinolysin, but has only minimum humoral defenses against the 2 heterologous streptolysins.

The titers of the specific antiserums suggest that only 6 (21%) of the 28 serums tested are of sufficiently high antifibrinolytic content to serve as effective passive antifibrinolytic immunizing agents in man.‡ But 2 (7%) of them would be similarly effective with horses. None of the antiserums would have a predictable antifibrinolytic immunizing value for domestic swine.

It is of interest that the 7 "refined" and "concentrated" anti-

* Work supported in part by the Eli Lilly and Co. Streptococcus Research Fellowship of Stanford University and in part by the Rockefeller Fluid Research Fund of the Stanford Medical School.

† These antiserums were kindly furnished by: Eli Lilly and Co.; The Cutter Laboratory; Parke, Davis and Co.; Lederle Laboratories; and E. R. Squibb and Sons.

1 Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

2 Madison, R. R., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 444; 1933, **32**, 641.

‡ So far as known there is no parallelism between the antifibrinolytic titer and therapeutic value of antistreptococcus serums. (W. H. M.)

TABLE I.

Titer of Streptococcus Antiserums.

An arbitrary dose of approximately 10 minimum fibrinolytic units of each streptococcus filtrate was added to 1:10, 1:100, 1:1000, and 1:10,000 dilutions of each antiserum, controls being run with normal human, horse, and swine serum. The resulting mixtures were incubated for 3 hours, then tested for their fibrinolytic power. Purified human, horse, and hog fibrins were used in all fibrinolytic tests, the technic throughout being identical with that used by Tillett and Garner.[1] The maximum serial dilution of a serum giving complete neutralization of the arbitrary fibrinolytic dose was taken as its approximate titer. 0, no demonstrable neutralization of the fibrinolytic dose in the highest serum concentration tested, *i. e.*, 1:10.

The table records composite data from 2 tests with different streptococcus filtrates. In several cases the 2 tests gave slightly different titers with the same serum. In such cases the mathematical average is recorded.

	Antifibrinolytic units per cc. when titrated against the arbitrary dose of:		
Serums	Anti-human streptofibrinolysin	Anti-horse streptofibrinolysin	Anti-swine streptofibrinolysin
Normal Serum Controls:			
Pooled adult human serum A, B, C	50	5	10
Normal horse serum A, B	0	100	5
Normal hog serum A, B	5	10	50
Commercial Antiserums:			
Nos. 23, 30, 31	0	100	5
22	0	1000	5
25§	5	500	10
11	5	1000	5
29	10	100	0
14, 19	10	1000	0
7, 9, 10	10	1000	10
21	50	100	10
16	100	50	5
18	100	500	0
13	100	1000	0
24§, 32§, 33§	100	1000	5
1, 15§	100	1000	10
5	100	5000	5
20§	1000	100	5
17	1000	1000	5
2	1000	1000	10
6§, 12	1000	1000	100
8	1000	5000	50

§ "Refined" and "concentrated" antiserums.

serums in the above list are not appreciably superior to the 21 presumably untreated antiserums.

7995 C

Comparison of Resistance of Bacteria and Embryonic Tissue to Germicidal Substances. IV. Hexylresorcinol.

A. J. SALLE AND A. S. LAZARUS.

From the Department of Bacteriology, University of California, Berkeley.

Leonard[1, 2] in his studies on some alkyl derivatives of resorcinol reported excellent results from hexylresorcinol when used as a urinary disinfectant. He stated that, "Hexylresorcinol, a stable organic substance of known chemical constitution, is the most powerful germicide ever described as a non-toxic substance. Hexylresorcinol is non-toxic by mouth and is administered in repeated doses for indefinite periods." Leonard and Wood,[3] Leonard and Frobisher,[4] Frobisher[5] and Leonard and Feirer[6] found that hexylresorcinol was a powerful surface tension depressant and that its remarkable germicidal property was probably dependent upon this physical property.

In previous papers of this series[7, 8, 9] comparisons were made of the resistance of *Staphylococcus aureus* and embryonic chick heart tissue to Merthiolate, Metaphen, Mercurochrome and phenol. A *Staphylococcus aureus* phenol coefficient and a toxicity index were determined for each germicide. The methods followed were the same as those described in the first communication.[7]

The highest dilution of phenol required to kill *Staphylococcus aureus* in 10 minutes but not in 5 minutes was 1:65. For Hexylresorcinol it was 1:7,000. This gave Hexylresorcinol a *Staphylococcus aureus* phenol coefficient of 108.

Leonard,[1, 2] Leonard and Wood[3] and Leonard and Feirer[6] reported *Staphylococcus aureus* phenol coefficients ranging from 46 to 72 when tested by other methods. Leonard[1, 2] stated that in an acid urine (pH 6.0-6.4) Hexylresorcinol in a dilution of 1:60,000

1 Leonard, V., *J. Urol.*, 1924, **12**, 585.

2 Leonard, V., *J. A. M. A.*, 1924, **83**, 2005

3 Leonard, V., and Wood, A., *J. A. M. A.*, 1925, **85**, 1855.

4 Leonard, V., and Frobisher, M., *J. Urol.*, 1926, 15, 1.

5 Frobisher, Jr., M., *J. Bact.*, 1927, **13**, 163.

6 Leonard, V., and Feirer, W. A., *Bull. Johns Hopkins Hosp.*, 1927, **41**, 21.

7 Salle, A. J., and Lazarus, A. S., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 665.

8 Salle, A. J., and Lazarus, A. S., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 937.

9 Salle, A. J., and Lazarus, A. S., PROC. SOC. EXP. BIOL. AND MED. (In press.)

killed *Staphylococcus aureus* in 1 hour and a 1:70,000 dilution killed in 24 hours. In an alkaline urine (pH 7.6-8.2) a 1:18,000 dilution of the germicide killed *Staphylococcus aureus* in 1 hour; a 1:60,000 dilution killed in 24 hours. Leonard and Feirer[6] found that a 30% solution of glycerin in water, containing 1 mg. of Hexylresorcinol per cc. was a very effective germicidal solution. A 1:8,000 dilution of this preparation killed *Staphylococcus aureus* in 15 seconds; a 1:9,000 dilution killed in 30 seconds. From our results it is concluded that Hexylresorcinol is a powerful germicide, having a *Staphylococcus aureus* phenol coefficient of 108 when tested by the method of Reddish.

The tissue culture results are summarized in Table I.

TABLE I.

Germicide	Highest Dilution Showing No Tissue Growth = A	Highest Dilution Showing No Growth of *Staphyl. aureus* = B	Toxicity Index = A/B	*Staphylococcus aureus* phenol coefficient
Phenol	1-840	1-65	12.9	
Hexylresorcinol	1-21000	1-7000	3.0	108

It is seen that the Hexylresorcinol is relatively very non-toxic and that it rated considerably higher than any of the germicides so far studied when tested by the tissue culture method. Also it gave a higher *Staphylococcus aureus* phenol coefficient. The germicides may now be placed in the following order on the basis of their toxicity indices: Hexylresorcinol 3.0; Metaphen 12.7; phenol 12.9; Merthiolate 35.3; and Mercurochrome 262.0.

Illinois Section

Northwestern University Medical School, March 28, 1935.

7996 C

Non-Production of Granulocytopenia with an Amidopyrine Compound in Some Acute Infections.

M. M. KUNDE, R. P. HERWICK, A. LEARNER, AND MARTIN STERNBACK.

From the Departments of Physiology, Pharmacology, and Pathology of the Chicago Medical School.

The experimental production of Agranulocytosis in rabbits by subcutaneous injections of benzene and olive oil was reported by Kracke.[1] Later Madison and Squire[2] emphasized the fact that in their analysis of 13 consecutive cases of granulocytopenia, all of the patients had received drugs containing barbiturate compounds with amidopyrine. Since then numerous reports[3] by physicians seem to indicate that amidopyrine is an important factor in the production of granulocytopenia. However, the attempts to produce granulocytopenia with amidopyrine have not been very gratifying. Madison and Squire's observation of an anti-mortem drop in the granulocytes of 1 rabbit in a series of 11 experimental animals receiving amidopyrine with allylisopropyl-barbituric acid, may have unduly suggested the possibility of a specific agranulocytic property of this drug, thus leading to erroneous conclusion as to the real cause of the disappearance of the granulocytes and placing a ban on the use of drugs hitherto considered relatively free from untoward symptoms, and very effective as analgesics and antipyretics.

The present speculative nature, both as to the real etiology of granulocytopenia and the rôle that these drugs play in this disease give evidence of a timely need for more experimental investigation and accurate clinical observation, which may further our information on these obscure but significant factors.

---

1 Kracke, Roy R., *Am. J. Clin. Path.*, 1932, **2**, 11.

2 Madison, F. W., and Squires, T. L., *J. A. M. A.*, 1933, **101**, 2076.

3 Reznikoff, Paul (Special Report of the Council on Pharmacy and Chemistry), *J. A. M. A.*, 1934, **102**, 2183; Johnson, Wingate M., *J. A. M. A.*, 1934, **103**, 1299.

The following experiments were performed at a time when 2 acute diseases were endemic among the rabbits of our laboratory, and occurred at frequent and irregular intervals, *viz.*, (1) an acute respiratory infection commonly called snuffles, from which rabbits frequently recover spontaneously and (2) an acute intestinal disturbance characterized by diarrhoea, loss in weight, and death. Before beginning the experiments we observed that rabbits infected with either of these diseases develop a pronounced leucocytosis.

The plan of our experiment consisted in administering cibalgine "Ciba" [Amidopyrine (dimethyl-amino-phenyl-dimethyl-pyrazolone) and dial (diallyl malonyl urea)] by mouth, in large daily dosages, to rabbits housed in adjacent cages, or in the same cage with infected rabbits, and comparing the leucocyte count of those which became spontaneously infected while receiving the drug, to those which received the drug but escaped infection.

Thirty-five rabbits were used. Nine of these were thyroidectomized 4 to 6 weeks before beginning the use of the drug. The object of the thyroidectomy was to impair the activity of the bone marrow; it having been previously shown that thyroidectomy in the rabbit is followed after a period of time by a histological picture of the bone marrow suggestive of damage to this tissue (Kunde[4]). The experimental production of such a bone marrow seems pertinent at this time, inasmuch as it has been generally considered that individuals with granulocytopenia have a defective bone marrow.

Table I contains a summary of the data taken from our protocols. The 35 rabbits used were divided into 7 groups. Group A consisted of 9 normal animals. Thirty-nine leucocyte counts were made of this group (3-6 counts on each animal always on different days) to establish variations in the number of leucocytes occurring in rabbits under normal conditions, apparently free from infection, and receiving no drug. The variation in number of leucocytes ranged from 3,200 to 10,536 per c.mm. with an average of 6,887. It is our opinion at this time, however, that the leucocyte count in normal young rabbits entirely free from infection rarely exceeds 6,000 and that counts exceeding this are indicative of mild infection giving no detectable gross signs. Group B represents a comparable group of normal animals, *i. e.* apparently free from infection but receiving dosages of the compound (1 to 4 tablets) daily, over a period of time 7 to 17 days in duration to establish the effect of this compound on the leucocyte count of normal rabbits. In this group, the variation in number of leucocytes ranges from 8,938 to 17,575 per

[4] Kunde, M. M., *et al.*, *Am. J. Physiol.*, 1932, **99**, 469.

c.mm. These results clearly indicate that large dosages of the compound caused no suppression of leucocytes in normal rabbits when administered for 17 consecutive days.

Groups C and D consisted of infected animals. In Group C the infected animals received no drug whereas in Group D, the infected animals received the compound daily. No rabbits in these groups weighed more than 1100 gm. and varied in age from 3 to 4 months. A significant leucocytosis always preceded gross signs of either disease in the infected animals and as the severity of the disease progressed and became grossly obvious, the leucocytes frequently exceed 27,000 per c.mm. In the infected animals of Group D the compound was administered daily in dosages varying from 1 to 2 tablets for 21 to 30 days. Leucocyte counts made on the 20th to the 30th day of drug administration in infected animals revealed a leucocytosis of more than 34,000, showing that the drug has no tendency to depress the leucocytosis which accompanies these infections although it was administered in relatively large dosages for 21 to 30 days, at which times the animals died. Groups E, F and G were made up of rabbits with thyroids removed 3 to 4 weeks after birth. The leucocyte counts of these animals were made 4 to 6 weeks after extirpation of the thyroids. The data of the rabbits of Group E (Table I) gave no evidence of infection. They received no drug. The data show that in this stage of thyroid deficiency (4 to 6 weeks after thyroidectomy) with no infection, the range of variation in leucocytes is approximately the same as in normal rabbits. In Group E, the thyroidectomized rabbits became infected. Infection in these thyroidectomized rabbits produced a severe leucocytosis, as occurs in infected rabbits with thyroids intact. The rabbits of Group G, as in Group E, were thyroidectomized and infected but these animals received large daily dosages (1-4 tablets) of the compound (10 to 22 days). In the rabbits of this group, the infection was characterized by a severe leucocytosis (more than 26,000 per c.mm.) comparable to that which occurs in infected animals receiving no drug.

Many differential counts were made from time to time, of animals to which the drug was administered. The percentage of granulocytes of the total leucocyte count was at all times within range of normal variations.

Summary. Cibalgine (Ciba) does not diminish the number of leucocytes in the circulating blood of normal rabbits when given in large dosages for 17 consecutive days. The pronounced leucocytosis which occurs in rabbits with sniffles or a certain gastro-

TABLE I.
Summary of Leucocyte Counts under Conditions of This Experiment.

Condition of animal	No. of animals	No. of counts	No. of counts each animal	Daily dose in *Tablets	No. of daily dosages	Leucocyte Counts			Remarks
						Lowest	Highest	Average	
A Thyroid intact. No drug, no infection. (Normal)	9	39	3-6	—	—	3,200	10,536	6,887	Normal animals
B Thyroids intact. No infection. Rec. drug daily	6	15	2-3	1-4	7-17	8,938	17,575	13,306	Killed between the 7 and 17 day.
C Thyroids intact. No drug. Infected.	6	12	2	—	—	11,150	27,200	20,180	Died either of respiratory or intestinal infection.
D Thyroids intact. Infection. Rec. drug daily.	3	15	3-6	1-2	21-30	6,650	34,550	12,835	Died either of respiratory or intestinal infection.
E Thyroids removed. No drug. No infection.	3	15	3-9	—	—	6,200	9,408	7,577	Uncomplicated hypothyroidism
F Thyroids removed. No drug. Infected.	4	9	2-3	—	—	8,350	26,989	19,207	Died either of respiratory or intestinal infection.
G Thyroids removed Rec. drug daily Infected.	4	12	1-4	1-4	10-22	7,712	26,500	13,544	Died either of respiratory or intestinal infection.

* Each tablet contains:
Amidopyrine (Dimethy-amino-phenyl-dimethyl-pyrazolone.....3½ grains
Dial, "Ciba" (diallylmalonylurea)..... ½ grain

intestinal infection is not depressed by the above mentioned amidopyrine barbiturate compound administered from 17 to 30 days. Rabbits thyroidectomized 6 weeks previously and receiving cibalgine develop a leucocytosis in response to the infections comparable to that which occurs in rabbits with thyroids intact.

7997 P

Isolation of Bacterium Typhosum When Mixed With Anaerobic, Non-Spore Forming, Gram-Negative Rods (Bacteroides)

SARAH J. BARRINGER AND G. M. DACK.

From the Department of Hygiene and Bacteriology, University of Chicago.

The enormous numbers of anaerobic non-spore forming, gram-negative rods reported in the intestinal contents of man (Sanborn[1] and Eggerth and Gagnon[2]) have not been considered in the routine isolation of pathogenic bacteria, or in the false presumptive tests in water analyses. *Bacteroides* are small gram-negative or gram-positive rods, some of the gram-negative forms being indistinguishable morphologically from the gram-negative pathogenic bacteria. Although they are strict anaerobes, the possibility that they might find conditions favorable for multiplication in mixed cultures with aerobes was considered in this investigation.

Bacterium typhosum (Rawlings) and 2 strains of *Bacteroides,* one isolated from a colon specimen from man and the other from a monkey colon, were studied. The biochemical reactions of the 2 strains of *Bacteroides* are listed in Table I.

TABLE I.

Strain	Gas from peptone	Gas from glucose	Glycerol	Mannitol	Sorbitol	Arabinose	Salicin	Trehalose	Amygdalin	Cellobiose	Glycogen	Rhamnose	Xylose	Lactose	Levulose	Glucose	Gelatin liquefaction	Milk	Indol	Lead acetate
Monkey origin	—	—	—	—	—	a	—	—	a	—	a	a	a	a	a	a	+	a	—	—
Human origin			—	—	—		—	—	a	a	a	—	a	a	a	a	—		—	—

— = negative test. a = acid production only. + = positive test.

1 Sanborn, A. G., *J. Infect. Dis.*, 1931, **48**, 541.

2 Eggerth, A. H., and Gagnon, B. H., *J. Bact.*, 1933, **25**, 398.

A mixed suspension of these *Bacteroides* strains and *Bacterium typhosum* produced acid without gas in lactose broth incubated aerobically. An experiment was performed with the human *Bacteroides* strain in mixture with *Bacterium typhosum*. This suspension was plated on endo and eosine-methylene blue agar media and incubated aerobically for 24 hours, after which colonies were picked and put into lactose broth. *Bacteroides* was demonstrated in a few of these colonies by the production of acid in the lactose broth after 36 hours' incubation. All of the colonies on the plates were colorless and identical with those of a pure culture of the typhoid bacillus, although plates prepared in a similar manner, incubated aerobically for 48 hours and then placed in an anaerobic jar and incubated for 3 days longer, contained some isolated colonies of *Bacteroides* and some which appeared to be superimposed on the typhoid colonies.

A mixed suspension of *Bacteroides* (monkey strain) and *Bacterium typhosum* was used for inoculation of 12 freshly prepared veal infusion agar slants. These were incubated aerobically at 37°C. Weekly transfers were made from these cultures to fresh slants. Lactose broth was inoculated to test for the presence of *Bacteroides*. In 4 of the 12 cultures, which were transferred at weekly intervals, *Bacteroides* remained viable for 4 weeks. In 5 it persisted for one week and in the remaining 3 it was not found after one week. Only one of these cultures was found to contain *Bacteroides* for 2 weeks without transfer.

An aerobic, 24-hour lactose broth mixed culture of *Bacteroides* and *Bacterium typhosum* in which the production of acid had just begun was used for inoculation of 10 more fresh veal infusion agar slants. These were handled in the same manner as the cultures above. In 8 of these cultures *Bacteroides* remained viable for 4 weeks when transferred at weekly intervals. In the other 2 they were found only after one week. Only one of these cultures contained *Bacteroides* for 2 weeks without transfer. The cultures in which *Bacteroides* remained viable for 4 weeks are still under observation.

A mixture of the human strain of *Bacteroides* and *Bacterium typhosum* was used for inoculation of eosine-methylene blue, endo's, veal infusion, and nutrient agar slants. *Bacteroides* remained viable for longer periods of time on eosine-methylene blue and endo's agar slants than on media which contained no carbohydrate. Results of these studies are recorded in Table II.

Bacteroides were found to grow in aerobic cultures with organisms other than *Bacterium typhosum*. Sucrose, lactose and dextrose

TABLE II.
Persistence of *Bacteroides* mixed with *Bacterium typhosum* in aerobic slant cultures.

Medium	No. of transfers	*Bacteroides* viable after	No. of days viable when transferred	No. of days viable when not transferred
Endo agar	15	13 transfers	25	11
Eosine-methylene blue	15	13 "	25	23
Veal infusion agar	10	8 "	16	12
Nutrient agar	10	8 "	16	11

broth inoculated with *Bacterium pyocyaneus* and *Bacteroides* showed rapid acid production without gas when incubated aerobically, although the strain of pyocyaneus used did not ferment these sugars. A mixed suspension of *Bacterium coli communis* and *Bacteroides* formed acid and gas in sucrose under the same conditions.

7998 C

Serum Colloid Osmotic Pressure in Normal Pregnancy.

WILLIAM J. DIECKMANN.

From the Department of Obstetrics and Gynecology, the University of Chicago, and the Chicago Lying-in Hospital.

A slight amount of edema occurs in many women during normal pregnancy. A marked edema or even an anasarca may occur in pre-eclampsia and eclampsia. No adequate explanation has been given for the cause of the edema in normal or toxemic pregnancy.

In the edema of certain types of nephritis, malnutrition, and in some cases of cardiac disease, the cause is found in an abnormally low serum protein concentration. Various studies indicate that edema is likely to occur if the concentration of the serum protein is less than 5.5 gm. % or if the albumin concentration is less than 2.5 gm. %. Dieckmann and Wegner,[1] as well as other investigators, have demonstrated that the concentration of the serum protein in normal pregnancy is at the lower limits of normal. Their average figures obtained from the same women during pregnancy and the puerperium are given in Table I.

The studies of Schade,[2] Govaerts,[3] and Verney[4] would seem to

1 Dieckmann, Wm. J., and Wegner, C., *Arch. Int. Med.*, 1934, **53**, 353.
2 Schade, H., and Mentschel, H., *Z. f. klin. Med.*, 1924, **100**, 370.
3 Govaerts, M., *Bull. Acad. roy. de med. de Belgique*, 1927, **13**, 356.
4 Verney, E., *J. Physiol.*, 1926, **61**, 319.

TABLE I.
Means for Serum Protein Concentration in Normal Pregnancy.

	—Ante-Partum, Weeks—			—Post-Partum, Days—			
	10 to 15	26 to 35	36 to term	2 to 6	10 to 15	18 to 26	8 to 17 weeks
Serum Protein gm. %	6.77	6.42	6.51	6.13	6.80	6.88	7.26
Probable Error	0.06	0.05	0.04	0.09	0.07		
Standard Deviation	0.41	0.40	0.47	0.65	0.57		
Number of Cases	23	26	56	25	31	10	10

indicate that the oncotic or colloid osmotic pressure of 1 gm. of albumin is 7.54 cm. of water and 1 gm. of globulin is 1.95 cm. of water. Wells and co-workers,[5] after an extensive study of their methods and comparisons with one devised by himself, conclude that their figures are too high. He stated that "the specific osmotic pressure of serum appears to be a linear function of the albumin concentration. Variations in the globulin concentration over a wide range produce no effect on the specific pressure at constant values of albumin." The formula for the colloid osmotic pressure derived by him is: $P = C\,(21.4 + 5.9A)$, where P is the osmotic pressure in millimeters of water, C is the total protein concentration and A is the albumin concentration, in gm. per 100 cc. He found a standard of error of $\pm$ 5%.

Runge and Kessler[6] stated that the oncotic pressure was normal in the first trimester of pregnancy. At about the fourth month it began to decrease and steadily decreased until term. Fluctuations occurred during labor and the early puerperium, but it was normal on the eighth postpartum day. Similar changes in the concentration of the serum protein occur and are probably the cause of the alterations in the oncotic pressure.

Kaboth[7] reported a decrease in the oncotic pressure in pregnancy, but his fluctuations coincided with the changes in the concentration of the proteins.

A number of women at different periods of pregnancy were selected. All were apparently normal and none had more than a slight pitting edema of the ankles. In a number, serum was also obtained after delivery. The serum protein, albumin and globulin fractions were determined,[8] and the oncotic pressure measured with

5 Wells, H., Youmans, J., and Miller, D., *J. Clin. Invest.*, 1933, **12**, 1103.

6 Runge, H., and Kessler, R., *Arch. f. Gynak.*, 1925, **126**, 45.

7 Kaboth, G., *Arch. f. Gynak.*, 1926, **127**, 170.

8 Dieckmann, Wm. J., *J. Lab. and Clin. Med.*, 1931, **16**, 513.

Wells'[9] method. The collodion membranes used by us were standardized in his laboratory.

TABLE II.

No. of Patient	Period of Pregnancy Weeks	Serum Protein gm. %	Albumin gm. %	Oncotic Pressure cm. of Water		
				Determined	Calculated	Difference
1	2 mos. P.P.	7.18	4.17	31.5	33.0	—1.5
2	34	6.05	3.14	27.0	24.2	+2.8
	7 days P.P.	7.51	3.97	36.0	34.5	+1.5
3	33	6.57	3.22	29.1	26.7	+2.6
	8 days P.P.	7.33	3.79	32.6	32.8	—0.2
4	36	6.93	4.15	30.4	31.8	—1.4
	8 days P.P.	6.85	3.55	29.9	29.5	+0.4
5	20	6.34	3.44	26.7	26.5	+0.2
	20	6.90	3.67	29.6	30.0	—0.4
	7 days P.P.	6.82	3.55	31.1	29.0	+1.1
6	41	6.62	3.52	28.6	27.9	+0.7
	8 days P.P.	7.30	3.68	33.8	32.0	+1.8
7	40	6.29	3.27	25.2	25.8	—0.6
	7 days P.P.	7.33	4.02	34.1	33.5	+0.6
8	40	6.68	3.17	25.2	27.	—1.8
	8 days P.P.	7.50	4.40	34.7	36.	—1.3
9	40	7.02	3.91	27.8	31.5	—3.7
	11 days P.P.	7.21	3.87	32.5	32.0	+0.5
10	39	6.13	3.06	22.6	24.5	—1.9
	6 wks. P.P.	6.84	4.27	38.3	32.0	+6.3
11	33	6.84	4.16	30.0	31.5	—1.5
	40	7.36	4.58	31.0	35.5	—4.5
12	10	6.55	4.13	29.9	30.0	—0.1
13	37	7.04	4.25	33.3	33.0	+0.3
14	5 days P.P.	5.73	2.78	24.3	22.0	+2.3
15	40	6.87	3.49	29.8	29.0	+0.8
	7 days P.P.	6.78	3.19	27.7	27.5	+0.2
16	8	7.20	4.06	38.1	33.0	+5.1
17	34	6.70	3.93	27.9	30.0	—2.1
	7 days P.P.	6.84	3.22		28.	

P.P. = post-partum.

Our results are listed in Table II. The figures under the "Difference" column indicate, according to the sign, whether the determined measurement is greater or less than the calculated oncotic pressure. It is evident that with but few exceptions the determined and calculated pressures check within the limit of error.

We believe that this work indicates that in normal pregnancy there is no intrinsic change in the serum protein and that the edema is not due to a subnormal colloid osmotic pressure.

[9] Wells, H., *Tenn. Acad. Sci.*, 1933, **8**, 102.

7999 P

Total Ascorbic Acid Content of Human Blood.

I. ARTHUR MIRSKY, S. SWADESH, AND S. SOSKIN.

From the Metabolic Laboratory, Department of Physiology, Michael Reese Hospital.

The fact that generalized infections are more common in patients with a low tissue ascorbic acid content and that ascorbic acid may be related to this and other tissue phenomena have led to the suggestion that the determination of the ascorbic acid content of blood might be of diagnostic value.[1] We wish to report some values obtained in normal and pathologic patients by a method slightly modified from that of Emmerie and Van Eekelen.[2] This method determines the total content of ascorbic acid present in the reduced form, any oxidized material being converted to the reduced form by the procedure. We used 5 cc. instead of 10 cc. of blood and were able to increase the ease of obtaining satisfactory duplicate determinations by using acetic instead of trichloracetic acid for the final titration.

Our procedure is as follows: To 5 cc. of oxalated whole blood in a 50 cc. Erlenmeyer flask, add 5 cc. of 10% trichloracetic acid, shake and then add 5 cc. of 16.6% mercuric acetate; mix thoroughly and allow to stand for 5 minutes. Then add about 0.25 gm. calcium carbonate and mix until neutral to Congo Red. Transfer to a 15 cc. test-tube and centrifuge. Without decanting, allow H_2S to bubble through the supernatant solution for a few minutes and then filter into another 15 cc. test-tube. Through this filtrate again bubble H_2S until all the air in the tube is displaced; then stopper the tube and allow the solution to remain overnight in contact with the gas in the tube. On the following day bubble nitrogen through the solution for 15 minutes in order to remove the H_2S.

The 2:6 sodium dichlorphenolindophenol used for titration of the ascorbic acid is extracted with hot water (about 25 mg. in 50 cc.) and diluted until 12 cc. of this solution is equivalent to 1 mg. ascorbic acid as determined by standardization with a solution of pure ascorbic acid. To 5 cc. of the H_2S-free filtrate, 1 cc. of 10% acetic acid is added; the solution is mixed and titrated into a tube containing 0.1 cc. of the indicator solution. For the titration we have found it convenient to use a 1.0 cc. pipette calibrated in one-

1 Yavorsky, M., Almaden, P., and King, C. G., *J. B. C.*, 1934, **106**, 525.

2 Emmerle, A., and Van Eekelen, M., *Biochem. J.*, 1934, **28**, 1153.

TABLE I.

Amount of Ascorbic Acid added to 100 cc. blood mg.	Ascorbic Acid found per 100 cc. blood mg.	Recovery %
0	1.68	—
1.2	2.92	105
1.8	3.33	93

hundredths. The titration should be complete within 3 minutes. This procedure allows for sufficient filtrate to permit 2 titrations. Table I indicates the degree to which added ascorbic acid may be recovered from blood.

Allowing the blood to stand at room temperature for varying periods up to 24 hours has no appreciable effect on its ascorbic acid content.

All figures on which this report is based were obtained from bloods taken in the post-absorptive state and represent observations on about 100 different individuals. The values in apparently normal individuals ranged from 1.19 to 2.66 mg. %. Those in patients suffering from a variety of chronic diseases (including diabetes mellitus, hyperthyroidism, rheumatic heart disease, arteriosclerosis, acromegaly and chronic glomerular nephritis) ranged from 1.11 to 2.88 mg. %. No correlation between the diseases investigated and the ascorbic acid values obtained is apparent, although cases of coronary sclerosis were almost uniformly grouped at the upper limit of the above range. We have also been unable to make any correlation between the total ascorbic acid content of the blood and the dietary regime.

8000 P

A Biliary Precipitate Characteristic of Cholelithiasis.

I. ARTHUR MIRSKY. (Introduced by Samuel Soskin.)

*From the Metabolic Laboratory, Department of Physiology, Michael Reese Hospital.**

We have been unable to find any previous report of a chemical test by which bile drawn from the gall-bladder or bile ducts of patients suffering from cholelithiasis may be distinguished from

* This work was started at the Richard Morton Koster Laboratory, Brooklyn, New York.

normal human bile. For the past 3 years we have been observing a phenomenon, as yet unexplained, which is characteristic of the bile drawn by needle and syringe from human gall bladders or bile ducts containing calculi, but which is absent in normal human bile and in dog's bile. Through the courtesy of Dr. Harry Koster of Brooklyn, N. Y., and of Dr. Ralph Bettman of Chicago, Illinois, we have now observed this reaction in over 200 cases of cholelithiasis. Our attempts to make this test on biles obtained by duodenal drainage have been unsuccessful thus far, due probably to the factors of dilution and interfering substances. We are reporting our observations at this time, in the hope of stimulating work by others which may lead to a valuable clinical test for cholelithiasis.

The characteristic phenomenon is observed when 1 cc. of N/12 sulphuric acid is added to 1 cc. of fresh bile, removed by needle and syringe at operation, or autopsy soon after death, from patients with cholelithiasis. An immediate precipitation of a pale, yellow-green, gelatinous substance occurs. This precipitate, once seen, will not be confused with the fine, particulate precipitate produced by the same procedure in biles free from calculi. The amount of the characteristic precipitate which appears is roughly proportional to the bulk of the stones found in the gall-bladder, and our predictions in this regard, based on tests of biles without preceding knowledge of the surgical pathology, have been surprisingly accurate. We have been unable to make any correlation between the occurrence of the characteristic precipitate and the concentration, viscosity, protein, or cholesterol content of the bile which we tested.

In none of the 200 cases where gall stones were demonstrated did we fail to get the characteristic precipitate. However, in several cases, clinically diagnosed as cholelithiasis and in which the bile yielded a small amount of the typical precipitate, no calculi could be found. These instances may represent failures of the test, but probably do not in view of the following considerations. In one such case, careful examination of the gall bladder after surgical removal revealed a quantity of calculous material resembling sand. In another instance clinically diagnosed as cholelithiasis and in which the bile was positive according to our test, no stones were found at operation. Nevertheless a cholecystectomy was performed and a drainage tube inserted into the common bile duct. The bile collected from the drain continued to give consistently positive results and on the fourth day post-operative, a small calculus, presumably hepatic in origin, was passed through the drainage tube. This latter case also suggests that the substance responsible for the positive reaction

originates in the liver and not the gall bladder. This is confirmed by the fact that fluids drawn from the gall bladder in 2 cases of hydrops of the gall bladder with stones in the cystic duct, both failed to yield the characteristic reaction.

Our results indicate a significant difference from the normal in the bile from cases of cholelithiasis. The alteration in the composition of the bile responsible for the precipitate probably does not originate in the gall bladder. Further work on this problem should shed light on the origin of gall stones.

It is a pleasure to acknowledge the aid and direction of Dr. Samuel Soskin.

8001 C

Peptic Ulcers Produced by Feeding Cincophen to Mammals Other than the Dog.

S. O. SCHWARTZ AND J. P. SIMONDS.

From the Department of Pathology, Northwestern University Medical School.

Churchill and Van Wagoner[1] demonstrated that gastric and duodenal ulcers can be regularly produced in dogs by administration of cincophen. Cases of alleged cincophen poisoning in man have not been characterized by such ulcers. It appeared desirable, therefore, to determine whether this effect in the dog is a species-selective phenomenon.

Cats, rabbits and guinea pigs were given cincophen orally suspended in cotton seed oil. The dosages were calculated according to the principle used by Churchill and Van Wagoner, *i. e.*, on the basis of 22 mg. per kg. body weight which corresponds to the human dose of 7.5 grains t.i.d. for the average adult of 150 lbs. During the experiment the animals received the usual care given to the particular type of experimental animal. In each case the post-mortem examination was done as soon after death as possible.

The following tables give a resumé of the results.

Results with Cats

Cat No. 1. 2 doses each 10x N.H.D.* Death on second day. A few superficial erosions in gastric mucosa up to 4 mm. in diameter.

---

1 Churchill and Van Wagoner, PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 581; *Arch. Path.*, 1932, **14**, 860.

* N.H.D. Normal Human Dose.

Cat No. 2. 4 doses each 10x N.H.D. Death on 5th day. Numerous erosions and one ulcer 1.5 cm. in diameter, chiefly in the fundus of the stomach.

Cat No. 3. 12 doses each 5x N.H.D. Death on 14th day. Two superficial ulcers 4 and 7 mm. respectively, on lesser curvature near pylorus.

Cat No. 4. 17 doses each 2½x N.H.D. Death on 17th day. No ulcers in stomach.

Cat No. 5. 47 doses each 1x N.H.D. Death from peritonitis on 54th day. Perforating ulcer 3x10 mm. near the cardia. Several superficial ulcers up to 4 mm. on lesser curvature.

Cat No. 6. 62 doses 1x N.H.D. Death on 67th day. No ulcer. Large intestine contained several superficial ulcers and was edematous and hyperemic.

Results with Rabbits

Rabbit No. 1. 7 doses each 10x N.H.D. Death on 8th day from bronchopneumonia. No ulcers.

Rabbits Nos. 2 and 3. 87 doses each 10x N.H.D. Sacrificed on 99th day. No ulcers.

Rabbit No. 4. 31 doses each 25x N.H.D. Death on 35th day from bronchopneumonia. No ulcers.

Rabbit No. 5. 66 doses each 25x N.H.D. Sacrificed on 76th day. No ulcers.

Results with Guinea Pigs

Guinea Pig No. 1. 14 doses each 10x N.H.D. Death on 17th day. No ulcers.

Guinea Pig No. 2. 19 doses each 10x N.H.D. Death on 68th day. No ulcers.

Guinea Pig No. 3. 11 doses each 10x N.H.D. Death on 15th day. No ulcers.

Guinea Pig No. 4. 15 doses each 5x N.H.D. Death on 17th day. No ulcers.

Guinea Pig No. 5. 90 doses each 2½x N.H.D. Sacrificed on 107th day. No ulcers.

Summary and Conclusions. 1. Cats were found to be very susceptible to the toxic effects of cincophen. They survived daily doses 10x N.H.D. from 2 to 5 days, and 1x N.H.D. for only 62 days. Four of the 6 cats used developed gastric ulcers, one of which perforated.

2. Rabbits are very resistant to cincophen. None of our animals died from the effects of the drug. One survived 66 doses, each 25x N.H.D., without apparent injury. None developed gastric ulcers.

3. Guinea pigs are moderately resistant to the toxic effect of cincophen. They withstood from 11 to 19 doses each 10x N.H.D. for 2 weeks or more, and one animal survived 90 doses each 2½x N.H.D. None developed gastric ulcers.

8002 C

Complement Titer of the Blood in Allergic Conditions.

EVELYN B. TILDEN. (Introduced by Arthur I. Kendall.)

From the Department of Research Bacteriology, Northwestern University Medical School.

Titration of the complement of the blood serum has been recommended for the diagnosis of liver disease,[1] rheumatic affections,[2] and yellow fever,[3] and recently it has been stated,[4] on the basis of a single case report by Deutsch and Weiss,[5] that complement titer is markedly lowered in allergy. Inasmuch, therefore, as no considerable number of titrations appears to have been carried out on the blood of typical cases of allergic disease, *i. e.*, hay fever and asthma, it seemed desirable to study a series of such cases.

The sera from allergic patients were kindly furnished by Dr. S.

TABLE I.

Case No.	Type of allergy	Symptoms and Treatment	Complement Titers*
1	Hay fever	Active symptoms, beginning treatment	.05
2	" "	" " " "	.05
3	" "	" " " "	.07
4	Asthma	Chronic, under treatment	.05
5 (9/22/34)	Hay fever	Active symptoms, under treatment	.07
6	Asthma	Chronic, had been treated a long time	.02
7	"	" " " " " " " "	.05
8	"	" under treatment	.05
9	"	Well at time of test	.07
10	"	Chronic, under treatment	.05
11	"	Comparatively well	.07
12	"	Chronic, beginning treatment	.05
5 (12/8/34)	Hay fever	Active symptoms, under treatment	.05
13	Asthma	Chronic, treated for a long time	.05
14	Urticaria		.07
15	Asthma	Chronic, under treatment	.05
16	"	" " "	.05
17	Hay fever	Not active	.07
18	Asthma	Chronic, well for 2 yrs., recurrence 1 month before test	.05
		Average	.05

* Cc. of serum required to hemolyze 1 cc. of 1% sheep cells in the presence of 2 units of antisheep amboceptor.

1 Bergel, A., and Schüle, F., *Wien. klin. Woch.*, 1931, **44**, 1562; **45**, 53.

2 Buchholz, B., *Deut. Arch. Klin. Med.*, 1934, **176**, 330.

3 Costa Cruz, J. da, and Villela, C. G., *Compt. rend. Soc. de Biol.*, 1933, **112**, 915.

4 Schattenberg, H. J., and Harris, W. H., Proc. Soc. Exp. Biol. and Med., 1934, **31**, 1446.

5 Deutsch, F., and Weiss, E., *Med. Klin.*, 1933, **29**, 1402.

M. Feinberg, in charge of the Allergy Clinic, and the control sera were obtained through the courtesy of Dr. O. E. Hepler, of the Clinical Pathology Laboratory, who also supplied the sheep cells and amboceptor used in the titrations. The complement titrations were carried out on the same day as the routine Wassermann tests, and the cells and amboceptor used had been titrated with guinea pig complement. The control series comprised a large number of sera (more than a hundred), which were being used for other experiments.

Veil and Buchholz[6] found the complement titer of normal blood to range between 0.02 cc. and 0.06 cc., with an average value of 0.05 cc. A similar titer was found for the control sera of the present series (0.02 cc. to 0.07 cc., with an average of 0.05 cc.), and the allergic sera showed a similar range and similar average value (Table I).

Conclusion. The amount of complement present in the blood of allergic patients of this series (18 cases) was similar to that in the control sera from persons coming to the general medical clinic.

8003 P

A Study of Experimental Meningococcal Infection. I. Method.

C. PHILLIP MILLER. (With the technical assistance of Ruth Castles.)

From the Department of Medicine, University of Chicago, and the A. B. Kuppenheimer Research Foundation.

It was reported[1] that mice could be infected by intraperitoneal inoculation with small numbers of virulent meningococci suspended in a solution of mucin. The mucin used in those original experiments was a commercial product which is prepared from hog's stomach and marketed for the treatment of gastric ulcer. The strains of meningococci employed had been freshly isolated from the spinal fluids of patients suffering from epidemic meningitis, and of these strains the 2 which proved lethal in highest dilution were those which had been obtained under conditions that permitted initiation of the experimental infection in the mouse most quickly after aspiration of the spinal fluid.

When work on this problem was resumed after several months,

[6] Veil, W. H., and Buchholz, B., *Klin. Woch.*, 1932, **11**, 2019.

[1] Miller, C. Phillip, *Science*, 1933, **78**, 340.

the minimal infecting inocula proved to be much larger than they had been in the preceding spring and summer. To explain this variation, attention was directed to the meningococci, the mice, and the mucin. In the case of each lay a possible source of our difficulty because the following changes had occurred:

1. Our cultures had been carried for a considerable length of time on artificial media without transfer to mice. Efforts to enhance the virulence by mouse passage were unavailing, and fresh strains of meningococci could not be secured because of the low incidence of epidemic meningitis in Chicago at the time.

2. Mice were difficult to obtain and came mostly from dealers who had not previously supplied us; presumably, therefore, from different stocks.

3. A new lot of mucin was being used as our initial supply had been exhausted. Several lots obtained from the laboratory which had furnished it and samples from the 2 other manufacturers who prepare mucin commercially were equally ineffective in facilitating experimental infection with small numbers of organisms. At length, on the assumption that the process of purification might "denature" the mucin or destroy some unrecognized ingredient essential to our purposes, the laboratory which had originally supplied us was requested to make up a special lot of mucin.* This proved to be wholly satisfactory and was used in the experiments described below.

Preparation of the mucin suspension. A 5% suspension is made up as follows: Onto the weighed quantity of mucin is poured enough distilled water to moisten it thoroughly. It is allowed to stand half an hour. Then the sticky mass is stirred and rubbed free of solid lumps. Stirring continues as distilled water is slowly added until the final concentration of 5% is reached. The reaction of suspension should be about pH = 5. It is placed in suitable containers and autoclaved at 15 lb. pressure for 15 minutes. After cooling, the reaction is adjusted with normal sodium hydroxide to pH = 7.3 and glucose is added to a concentration of 1%. After incubating one day to insure sterility the suspension is ready for use. As the product now being used contains particles of sufficient size to occlude fine hypodermic needles, it has been found convenient to withdraw the supernatant from this coarse sediment which rapidly settles to the bottom of the container and is easily visible to the naked eye. The supernatant, hereinafter designated the "mucin

*For this material the author is indebted to Dr. David Klein of the Wilson Laboratories, Chicago. It may be obtained under the label "Granular Mucin."

suspension" tends itself to settle on standing, particularly in the cold, and should be thoroughly mixed by gentle agitation before using, in order to insure its uniform composition in all experiments.

Stock cultures of meningococci are maintained on solid media consisting of equal parts of meat infusion broth and buffer solution (containing 6.6 gm. of $Na_2HPO_4.12H_2O$ and 0.83 gm. of $NaH_2PO_4.H_2O$ and 8.8 gm. NaCl per liter), 1% dextrose, 1% peptone, 1% corn starch and 2% agar. Its reaction is pH = 7.3 to 7.4. Inoculated agar slants are kept stoppered with corks. Although stock cultures are transplanted only twice a week, a strain which is to be used for mouse inoculation is subcultured at least twice at daily intervals before the final culture is inoculated. The organisms to be used are grown on the same medium for only 5 hours. They are then washed off with a few cubic centimeters of saline, the suspension thoroughly mixed and diluted with saline to a density which experience has shown to contain approximately $5\text{-}10 \times 10^9$ organisms per cc. Their numbers are determined by the Wright method, the smears being made at once, but the actual counting postponed until the completion of the animal inoculations.

The bacterial suspension is then titrated by progressive ten-fold dilution in the suspension of mucin. Mice are injected intraperitoneally with 1.0 cc. amounts as soon as possible after the dilutions have been prepared in order that their estimated bacterial content shall be altered as little as possible either by multiplication or death.

By this method a lethal infection can be initiated in the mouse by the intraperitoneal inoculation of a very few organisms, with our most virulent strains, approximately 10.

8004 P

A Study of Experimental Meningococcal Infection. II. Course of Infection.

C. PHILLIP MILLER. (With the technical assistance of Ruth Castles.)

From the Department of Medicine, University of Chicago, and the A. B. Kuppenheimer Research Foundation.

Mice infected intraperitoneally by the method described in the preceding communication[1] rapidly sicken and die within a period of 12 to 48 hours, depending on the number of meningococci injected.

[1] Miller, C. Phillip, PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1136.

The cellular and microbic contents of the peritoneal cavities of the mice in several series were studied at intervals of about 2 hours. A drop of exudate was aspirated by means of a fine hypodermic needle inserted into the peritoneal cavity and examined microscopically in stained film preparation. As a check on this method the entire peritoneal exudate was withdrawn from mice sacrificed at corresponding times during the course of their infection and mixed and examined by means of cultures and smears. The results of these observations may be summarized as follows: Cells did not appear in the peritoneal exudate in any considerable number until about 4 hours after inoculation, even when very heavy suspensions of organisms had been injected. From that time on the number of cells increased rapidly. Approximately two-thirds of them were polymorphonuclear leucocytes and one-third large and small cells with a single nucleus and various amounts of cytoplasm. No special methods were employed to differentiate these cells. As the infection progressed an increasing fraction of the cells showed evidences of degeneration.

Following inoculation with small or moderately large numbers of meningococci evidence of multiplication was seldom obtained for several hours. After 4 to 6 hours, however, the number of organisms increased rapidly and apparently steadily until death occurred. When the infection was initiated by injection of relatively massive inocula the multiplication seemed to begin earlier. Irrespective of the number of organisms inoculated very few were seen within leucocytes at any stage of the infection when the strain employed was a highly virulent one. The exudates from mice infected with less virulent strains, however, contained an appreciably larger proportion of polymorphonuclears in which diplococci were visible. In other words, the extent to which phagocytosis occurred was apparently related to the virulence of the infecting organism rather than to the numbers of individuals introduced into the peritoneal cavity.

Invasion of the blood stream was found to occur very shortly after intraperitoneal inoculation with large numbers of organisms. This point was investigated by culturing on the surface of freshly poured blood-agar plates a drop of blood drawn into a fine capillary pipette from the freshly cut end of the tail. The method was checked by culturing the heart's blood of mice sacrificed from time to time during the course of their infection. When the intraperitoneal inocula exceeded a million organisms, cultures of blood drawn within 15 minutes were positive, and the bacteremia persisted until death. Inoculations of a few hundred thousand organisms were

followed by cultures which did not become regularly positive for one or 2 hours. Still smaller inocula failed to produce a sustained bacteremia for 4-12 hours, though occasionally a "weakly positive" culture (*i. e.*, one or 2 colonies from a drop of blood) was followed by several negative ones. In the case of mice infected with very few organisms occasional cultures were "weakly positive" early in the course of the infection. All cultures of the blood of moribund mice were "strongly positive", *i. e.*, showed many colonies or a confluent growth on the plates. In summary, therefore, these observations indicate that transitory invasion of the blood stream may occur at any time during the course of the infection, but that the time at which persistent bacteremia begins is related roughly to the number of organisms with which the infection is initiated.

8005 P

A Study of Experimental Meningococcal Infection. III. Effect of Anti-bacterial Immune Serum.

C. PHILLIP MILLER. (With the technical assistance of Ruth Castles.)

From the Department of Medicine, University of Chicago, and the A. B. Kuppenheimer Research Foundation.

The effect of immune sera on experimental meningococcal infection in the mouse was studied to determine: (a) its protective action, when administered before inoculation, and (b) its ability to alter the expected outcome of an infection when administered during its course.

Sera were obtained from rabbits immunized by intravenous injections of living meningococci grown on solid media. Therapeutic sera (most of them "concentrated") prepared by a number of different commercial firms were purchased in the open market.*

Preliminary experiments showed that serum administered subcutaneously afforded less protection than corresponding doses given intravenously or intraperitoneally, these 2 being equally effective. As the intraperitoneal route was much less time-consuming it was used in all experiments herein reported. It was thought for a time that the preservatives added to commercial therapeutic sera might

* In addition 2 lots of concentrated sera, one prepared with and one without preservative (Merthiolate), were very kindly supplied by Mr. W. A. Jamieson, Director, Biological Division of the Lilly Research Laboratories, Indianapolis.

exert a bacteriostatic action on the organisms injected into the peritoneal cavity 30 minutes later, but this supposition was proved to be unwarranted by the observation that the injection of such preservatives in concentrations considerably higher than those customarily employed failed to afford any detectable protection.

Dilutions of the sera were made with normal saline and were injected intraperitoneally in 0.5 cc. amounts approximately one-half hour before inoculation. Control mice received 0.5 cc. of saline at the same time. The inoculations were made by intraperitoneal injection of 1 cc. amounts of the suspensions of meningococci in mucin, titrated by ten-fold dilution, according to the method described in the first paper.[1] Although the weights of mice used varied from 18 to 24 gm., all of the animals in any given experiment weighed within 1 gm. of each other, a condition which placed a limitation on the size of the experiments.

Serum diluted 1:16 or less protected mice against inocula 100,000 to 10 million times the M.L.D. Serum diluted 1:2,000 protected against 100 to 10,000 M.L.D. Within this range protection was roughly proportional to the dilution of serum. Dilutions higher than 1:2,000 afforded inconstant protection against even small inocula. Among the few strains thus far studied, no evidence of strain specificity, as regards protection, has been encountered.

The results of protection experiments with commercial "concentrated" therapeutic serum indicate a degree of protection approaching, but not quite as high, as that effected by our rabbit serum.

To determine the duration of passive protection mice were injected with 0.5 cc. of quite a low (1:4) dilution of immune serum and tested, half of them 2 days, the rest 8 days later. They survived infection with an inoculum 100,000 times the lethal one.

The effect of immune serum on the experimental infection after its inception was studied by injecting the serum intraperitoneally into mice at different times after they had been inoculated with numbers of organisms which the controls showed to be varying multiples of a lethal inoculum. The results show that even relatively late in the course of the infection mice can be spared a fatal outcome by the administration of sizeable doses of serum.

Summary. Rabbit antimeningococcus immune sera as well as commercial therapeutic sera in high dilutions protect mice against infection with virulent meningococci. In comparatively low dilution they exert a favorable action on the course of the experimental in-

[1] Miller, C. Phillip. In press.

fection even when administered relatively late in its course. The mouse protection test is regarded as a more reasonable method than those at present employed for the standardization of therapeutic antimeningococcus sera.

8006 P

Experimental Freezing: Bleeding Volume, General and Local Temperature Changes.

HENRY N. HARKINS AND PAUL H. HARMON. (Introduced by Edmund Andrews.)

From the Douglas Smith Foundation, Department of Surgery, University of Chicago.

The bleeding volume in control dogs was found to be 58.6% of the calculated blood volume (one-thirteenth of the body weight) by Roome, Keith, and Phemister[1] and 53.4% by Harkins.[2] On the other hand, the former authors found that in shock due to trauma to an extremity, hemorrhage, plasmapheresis, and intestinal manipulation, the bleeding volume was greatly reduced, averaging 21.8% and in burns the latter author found it to average 20.3%. In another series of experimental burns it was found that in 6 burned animals in which the blood pressure was allowed to fall near a so-called shock level (50 to 82 mm. of Hg.) before the bleeding volume was determined, the bleeding volume averaged 26.3%. If, however, the bleeding was done before the blood pressure had fallen markedly (102 to 130 mm. of Hg.), the bleeding volume was already markedly reduced, averaging 31.4%.

The present work was undertaken to determine the bleeding volume in experimental freezing. Portions of the bodies of dogs were frozen by solid carbon dioxide under complete anesthesia (maintained till end of experiment) as described in previous papers[3] and the results shown in Table I. It is seen that in all instances the bleeding volume was below the normal values but the decrease was not quite as marked as in burned animals. In the first 3 experiments where the bleeding volume was only slightly reduced, the

[1] Roome, N. W., Keith, W. S., and Phemister, D. B., *Surg. Gynec. and Obstet.*, 1933, **56**, 161.

[2] Harkins, H. N., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 3.

[3] Harkins, H. N., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 432.

TABLE I.
Bleeding Volumes of Dogs with Experimental Freezing.

No.	Wt. Kg.	Interval from freezing to bleeding		Blood Pressure mm. Hg.		Hemoglobin, %		Hematocrit		Terminal bleeding vol. % cal. blood vol.
		Hr.	Min.	Start	End	Start	Max.	Start	Max.	
1	12.2	26	25	162	112	76	131	36	63	47.3
2	9.0	7	40	136	96	104	145	55	67	31.1
3	9.5	11	40	164	90	100	123	49	62	43.1
4	7.0	4	45	172	36	86	100	35	50	27.0
5	7.0	15	15	166	72	82	126	39	62	24.2
Aver.										34.5

bleeding was determined before the blood pressure had fallen to a so-called shock level.

In several of the frozen dogs very low general body temperatures were obtained. In one dog a rectal temperature of 25.7°C. (78°F.) was noted after 3 hours of constant application of solid carbon dioxide to practically the entire left side of the body. After removal of the ice, the temperature rose gradually to 33.7°C. (92°F.) at the time of death 16 hours later. This rectal temperature is not as low as that reported by Reincke[4] in 3 drunken men who had been exposed to cold. In these cases rectal temperatures of 27°C., 23 hours before death; 26.4°C., 13 hours before death; and 24°C. (75.2°F.) with recovery were reported.

Experiments on the rapidity of penetration of the cold were performed. It was found that living tissue was a much better insulator than dead tissue. At a depth of 2 cm. in one instance it took 17 minutes and in another 7 minutes for the body temperature to drop 10°C. (a 10 cm. cube of solid carbon dioxide being pressed tightly against the shaved skin). If the measuring thermometer was placed subcutaneously about 2 minutes' application was necessary to produce a 10°C. drop in temperature.

Summary. Experiments are reported to show that the bleeding volume is reduced in experimental freezing. General temperature studies in partially frozen animals were made and the low temperature of 25.7°C. (78°F.) reported in one instance. Local temperature studies were made to demonstrate the insulating function of living tissues.

4 Reincke, J. J., *Deutsch. Arch. f. klin. Med.*, 1875, **16**, 12.

8007 P

Bleeding Volume in Experimental Colon Bacillus Intoxication.

PAUL H. HARMON AND HENRY N. HARKINS. (Introduced by Edmund Andrews.)

From the Department of Surgery, University of Chicago.

The present investigation is an inquiry into the mechanism of the profound drop in blood pressure that follows the intravenous administration of bacterial-free filtrates of the colon bacillus *(Escherichia coli)*. Zinsser, Parker and Kuttner[1] and others[2, 3] demonstrated that this organism produced a soluble and filtrable toxic substance that caused death after an incubation period when injected intravenously. Harmon and Harkins[4] showed that a profound but delayed drop in blood pressure to a shock level occurred in dogs receiving bacterial-free filtrates by the venous route.

Roome, Keith and Phemister[5] pointed out that a low bleeding volume characterized secondary shock where irretrievable loss of plasma or whole blood from the vascular system occurred as in trauma to an extremity, hemorrhage, plasmapheresis and following intestinal manipulation, while primary shock as induced by hyperventilation, anaphylaxis, histamine administration, spinal cord section and spinal anesthesia was accompanied by a higher bleeding volume. Harkins pointed out that the shock that accompanies severe experimental burns[6] and freezing[7] is a secondary type of shock accompanied by a loss of fluid into the injured tissues resembling blood plasma. This same author demonstrated that the bleeding volume of animals in shock due to experimental burns[8] and to freezing[9] was low (averaging 20.3 and 34.5% of the calculated blood volume, respectively).

Bacteria-free filtrates of 7-day cultures of 4 strains of colon bacillus, grown in peptone-free veal infusion broth were prepared by

1 Zinsser, H., Parker, J. T., and Kuttner, A., PROC. SOC. EXP. BIOL. AND MED., 1920, **18**, 49.

2 Branham, S. E., *J. Infect. Dis.*, 1925, **37**, 538.

3 Steinberg, B., and Ecker, E. E., *J. Exp. Med.*, 1926, **43**, 443.

4 Harmon, P. H., and Harkins, H. N., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 6.

5 Roome, N. W., Keith, W. S., and Phemister, D. B., *Surg. Gynec. and Obstet.*, 1933, **56**, 161.

6 Harkins, H. N., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 994.

7 Harkins, H. N., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 432, 434.

8 Harkins, H. N., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 3.

9 Harkins, H. N., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 000

TABLE I.

Terminal Bleeding Volume: Associated Changes in Blood Pressure, Cell Volume, and Hemoglobin Following the Intravenous Injection of Colon Bacillus Filtrates.

Dog No.	Wt. Kg.	Cc. of Berkefeld filtrate intraven.	Time from inj. to death by bleeding (Hr.)	Blood Pressure mm. of Hg. Initial	Blood Pressure mm. of Hg. Prior to Bleeding	Hemoglobin, % Initial	Hemoglobin, % Prior to Bleeding	Hematocrit Initial	Hematocrit Prior to Bleeding	Terminal bleeding volume. % calc. blood vol.
1P	6	50 cc. *B. coli* Communis (Sw)	2.0	166	42	84	94	38	45	43
3P	7.5	50 cc. *B. coli* Communior (Di)	2.25	124	36	100	122	45	58	33
4P	5.0	50 cc. *B. coli* Communis (Ja)	8.25	164	40	91	105	46	50	61
5P	8.8	40 cc. *B. coli* Communior (La)	2.0	164	66	104	106	45	45	35
		Average								43

filtration through Berkefeld-N filters. Dogs from 5.0 to 8.8 kg. received a quantity of filtrate intravenously (Table I), after being under full anesthesia induced by barbital (275 mg. per kilo body weight). Blood pressure was recorded by the direct method from the carotid artery through a mercury manometer. Observations were made upon the percentage of hemoglobin (Sahli: 17 gm. per 100 cc. = 100%) and of the volume per cent of red cells with the Van Allen hematocrit both prior to injection of the filtrates and at intervals following intravenous injections. When the blood pressure declined to the shock level the bleeding volume was obtained by measuring the total volume of blood that escaped from the carotid artery with the animal tipped cephalad 15 degrees from the horizontal.

Results. Although the blood pressure dropped rapidly to the so-called shock level, there was but a minor degree of concentration of the blood as evidenced by only a slight rise in the percentage of hemoglobin in the circulating blood and in the hematocrit reading. The average bleeding volume in these experiments was 43% of the calculated blood volume. Although this result is lower than the average bleeding volume of 58.6% as found by Roome, Keith and Phemister[5] and of 53.4% as determined by Harkins,[8] it is definitely high enough to allow this type of shock to be classified as primary shock.

Conclusions. The shock state that follows intravenous injection of bacteria-free filtrates from the colon bacillus is classed as primary shock since the bleeding volume approximates that of the control animals.

New York Section

New York Academy of Medicine, April 17, 1935.

8008 P

Technique of Preparing Transplanted Uterine Fistulae.*

SANFORD KAMINESTER.† (Introduced by G. B. Ray.)

From the Department of Physiology, Long Island College of Medicine.

The purpose of the present paper is to describe a surgical preparation for studying the relationship between the extrinsic and intrinsic innervation of the rabbit's uterus and the myometrial response to oestrin and progestin. This method consists of transplanting the uterus to the anterior abdominal wall, thus severing all the pathways of the motor sympathetic nerves to this organ. It was necessary to perform the operation in 3 stages. After a brief experience it was found desirable to use animals that had dropped at least one litter. Adequate results can often be obtained in animals made pseudopregnant.

First stage. A mature female rabbit weighing approximately 3½ kg. was anesthetized with ether. A longitudinal incision was made to the right of the midline, starting about 3 cm. above the symphysis and extending cephalad a distance of about 6 to 8 cm. The right uterine cornu was brought up into the wound, the tubal end clamped, ligated and incised. Included in this ligature was the ovarian artery and whatever veins accompanied it. The free border of the uterus was then scarified as was an area on the peritoneal surface of the anterior abdominal wall. This area paralleled the original incision and was about ½ cm. lateral to it. The scarified area on the uterus was then brought into contact with that on the anterior abdominal wall and fixed there by means of interrupted sutures of plain catgut.

*Aided by a grant from the Committee for Research in Problems of Sex, of the National Research Council.

† From the Department of Obstetrics and Gynecology, Long Island College of Medicine.

These sutures penetrated about half the thickness of the muscle coat. Thus, this stage resulted in a ventro-fixation of the uterus.

Second stage. After an interval of from 1 to 3 weeks the second stage was undertaken. The abdomen was again opened, this time in the midline. The index finger of the left hand was passed over the cervical region of the uterus into the lateral gutter produced by the adhesions between the uterus and the anterior abdominal wall. In this manner the entire mesometrium was brought into view. This tissue was now incised and ligated with interrupted catgut suture-ligatures in order to sever the entire connection of the uterus from its original surrounding tissues except for the cervical attachment.

Third stage. After another interval of from 1 to 3 weeks the abdominal cavity was again entered. The vaginal tube was transected, care being taken to include all mesometrial tissue on the right side. The left uterine cornu was then cut across close to the cervix. As a result the uterus on the right terminated in a cuff of vagina containing 2 cervices, the transplant now being completely separated from the original contiguous tissues. Thus the uterus was dependent for its nutrition upon the vessels passing through the adhesions to the anterior abdominal wall and, occasionally, the intestines. The vaginal cuff was now brought up through the abdominal incision and anchored to the skin in such a way that the 2 cervices protruded so that a small balloon could be inserted for the recording of motility at some later time.

In some animals, at the beginning of the third stage the transplanted uterus was severed from its cervix and its lowermost portion brought up through the abdominal wall, resulting in a transplanted, cervicectomized uterus. Contractions could thus be recorded in the same way as in the non-cervicectomized uterus.

Occasionally localized infection in the operative region produced dense adhesions obliterating landmarks. Animals in which this difficulty was encountered were rejected since it could not be positively determined that all the mesometrium was severed. As a control, stimulation of the presacral nerves was used before sacrificing each animal. If a contraction of the transplant followed, the records obtained from that animal were rejected.

Occasionally, especially in the earlier animals, there was so much distortion of the transplant that it was difficult or impossible to insert a balloon.

The right ovary was removed at the time of the first stage, the left at the completion of the third in those animals in which ovariectomy was desired.

A detailed report of the application of this procedure and a study of motility in the rabbit's uterus under the influence of various hormones and drugs is now in the process of being completed.

8009 P

Innervation to and Within the Uterus.*

SAMUEL R. M. REYNOLDS AND SANFORD KAMINESTER.† (Introduced by George B. Ray.)

From the Department of Physiology, Long Island College of Medicine, Brooklyn.

In the course of certain experiments it became desirable to know the nature of the distribution of nerve paths from the pelvic plexus to their ultimate destinations in the uterus. Accordingly, we have performed a series of experiments in suitable ovariectomized rabbits. In some of these the parametrium was cut through progressively and the uterus left intact. In others, the uterus was cut first and the parametrium left entire. These experiments showed that when some spot in the middle third of the parametrium is cut, the uterus above the level of section fails to respond to lumbar sympathetic stimulation, even though the uterus itself is still intact. With the parametrium uncut, but the uterus severed, the whole organ still responds to pre-sacral nerve stimulation. Experiments on the essentiality of the utero-vaginal junction (containing the uterine cervical ganglia) were performed. This whole region, including cer vices, could be excised without modifying the responses of the uterine cornua when the lumbar sympathetic nerves are stimulated. Section of a selected small region of the parametrium at the level of and close to the site of the cervices, promptly abolishes uterine responses to sympathetic nerve stimulation.

In other experiments point stimulation of suitable places on the parametrium was made progressively from the tubal end, caudad. It was found in favorable experiments that the uterus contracts more or less locally, directly opposite the site of parametrial stimulation. This local contraction spreads very slowly in both directions, cephalad and caudad.

Our results indicate, therefore, that lumbar motor sympathetic

* Aided by a grant from the Committee for Research in Problems of Sex, of the National Research Council.

† Member of the Department of Obstetrics and Gynecology.

fibers pass close to the utero-vaginal junction and then dip more or less deeply into the parametrium as they pass toward the upper end of the uterus. Along their whole course they give off fibers to the uterus, innervating rather restricted regions, and do not contribute to a widespread, diffuse plexus within the uterine walls. This holds for the highly nervous utero-vaginal junction as well as for the uterus proper, which is virtually devoid of nerve cells.

8010 P

A Complex Polysaccharide Fraction from the Cells of the Human Type of Tubercle Bacillus, H 37.* †

MICHAEL HEIDELBERGER AND A. E. O. MENZEL.

From the Department of Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York, N. Y.

The recent work of Avery and Goebel[1] has shown the importance of omitting the use of alkali in the purification of the specific polysaccharide of Type I pneumococcus. It was accordingly deemed advisable to repeat the isolation of the tubercle bacillus fractions described in a recent note,[2] omitting the steps involving precipitation from alkaline solution, and keeping all solutions at least at a slight acidity. While the difficulties of the fractionation were increased, the B_1 and B_2 (low rotating) fractions were ultimately isolated in the same form as before. However, the C fraction (high rotating, insoluble in 75% methyl alcohol) differed markedly in its chemical properties. Aqueous solutions of the substance were opalescent, even after passage through a Berkefeld filter, and, on treatment with alkali, slowly deposited a bulky precipitate consisting mainly of magnesium palmitate. Magnesium was identified as the double ammonium phosphate, while the palmitic acid, crystallized from 60% methyl alcohol, melted at 53-57° and gave C = 75.18%, H = 12.73% (calculated, C = 74.92%, H = 12.59%), neutral

* Work carried out under a grant from the National Tuberculosis Association and with the aid of the Harkness Research Fund, Presbyterian Hospital, New York.

† Supplied through the courtesy of the H. K. Mulford Biological Laboratories of Sharpe & Dohme, Glenolden, Pa.

1 Avery, O. T., and Goebel, W. F., *J. Exp. Med.*, 1933, **58**, 731.

2 Heidelberger, M., and Menzel, A. E. O., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 631.

equivalent = 257, calculated 256. A portion of the acid was recrystallized, after which it melted at 56-59°. A mixture with highly purified palmitic acid melting at 60.5-61.5° fused at 57-59°. The polysaccharide recovered from the alkaline solution (CI) resembled the C fraction isolated by the usual process. The properties of the C and CI fractions are summarized in Table I, the data being calculated to the ash-free basis.

TABLE I.

Preparation No.	$[\alpha_D]$	Neutral equivalent	N %	P %	Acetyl %	Basic ash‡ %	Reducing sugars on hydrolysis§ %
520 C	+85°		0.15	0.39	2.7	1.0	93.3
520 CI	+84°	10000	0.05	0.10	1.7	0.0	

‡ As magnesium.

§ Hagedorn-Jensen method, giving 98.3%, calculated as glucose, with an earlier preparation corresponding to CI.

Both fractions reacted at a dilution of 1:2,000,000 with anti-H37 horse serum.[2]

While it is of course possible that the magnesium palmitate originates in substances accompanying the original polysaccharide, rather than as an integral portion of the polysaccharide complex, it is interesting to note that Boivin and his collaborators[3] have reported the isolation of a complex polysaccharide from *B. aertrycke* yielding an unidentified crystalline fatty acid on degradation. Landsteiner and Levene[4] also noted the occurrence of water-insoluble acids in the lipo-polysaccharide Forssman hapten.

8011 C

Bacteriological and Immunological Studies of Acute Glomerulonephritis in New Orleans.

B. C. SEEGAL, D. SEEGAL, E. L. JOST, AND P. CAMERON.

From the Departments of Bacteriology and Medicine, College of Physicians and Surgeons, Columbia University, the Presbyterian Hospital, New York City, and the Department of Medicine, Tulane University, New Orleans.

Previous work[1] has shown that the reported hospital medical admission rate for acute glomerulonephritis is similar in 4 latitude regions of the United States and southern Canada. In contrast,

3 Boivin *et al., Compt. rend. acad. sci.*, 1934, **198**, 2124.

4 Landsteiner, K., and Levene, P. A., *J. Immunol.*, 1925, **10**, 731.

1 Seegal, D., Seegal, B. C., and Jost, E. L., in press, *Am. J. Med. Sc.*

there is a diminished case frequency for scarlet fever[2] and rheumatic fever[3] in the South as compared with the North. Since clinical and laboratory evidence favors the hypothesis that acute glomerulonephritis is chiefly related to a preceding hemolytic streptococcus infection, it appeared peculiar that the diminution in incidence of the hemolytic streptococcus diseases, scarlet fever and rheumatic fever, in the southern latitudes did not also occur in the group of patients with acute nephritis.

In order to gain more accurate information concerning the type of infection predisposing toward acute glomerulonephritis in the South, a comparative study[4] of the hospital records of acute glomerulonephritis was made over a period of years in the following hospitals: The Presbyterian and Babies Hospitals in New York City; the Touro Infirmary, New Orleans; the John Sealy Hospital, Galveston; and the Baylor and Parkland Hospitals, Dallas. From this study it was concluded that the type of infection preceding the attack of acute glomerulonephritis was much the same irrespective of whether the cases occurred in the North or the South. The nature of the infection was that which is usually associated with tissue invasion by the hemolytic streptococcus. The majority of the patients in both groups had had such deep infections as acute cervical lymphadenitis, peritonsillar abscess, otitis media or acute mastoiditis prior to the onset of the acute nephritis. Study of the case histories in the southern hospitals, therefore, seemed to indicate that acute glomerulonephritis in the South, as in the North, resulted most frequently from a preceding hemolytic streptococcus infection. This could not be concluded with certainty since little bacteriological data on the incidence of the hemolytic streptococcus in the throats of healthy individuals and of patients with acute pharyngitis, peritonsillar abscess, lymphadenitis or mastoiditis are available in the cities of the South in which the case histories were studied.

In order to obtain specific data on the presence or absence of the hemolytic streptococcus in infections of the upper respiratory tract preceding or accompanying acute glomerulonephritis in the South, a visit to New Orleans was made in January and February of 1934 by two of us (D. S. and B. C. S.). Through the coöperation of Dr. Vidrine, Dr. Bass, Dr. Musser and Dr. Lemann we were enabled to study throat cultures from patients with acute glomerulonephritis, acute pharyngitis, non-streptococcus diseases, and from medical

2 Schroeder, H. A., and Longacre, A. F. Unpublished work.

3 Seegal, D., and Seegal, B. C., *J.A.M.A.*, 1927, **89**, 11.

4 Seegal, D., Seegal, B. C., and Lyttle, J. F., in press, *J.A.M.A.*

students at Tulane University Medical School. In addition blood was obtained from many of these individuals and the serum was later tested for the presence of the antistreptolysin of Todd. On our earlier visit to New Orleans we sought evidence for the presence of the hemolytic streptococcus in the South by means of testing intradermally a series of normal individuals with the nucleoprotein of the hemolytic streptococcus.

Throat cultures were taken by passing 2 swabs firmly over both tonsillar regions and the posterior pharynx. One of the swabs was then rubbed over about one-eighth of a rabbit blood agar plate* and the organisms further distributed with the platinum loop. The other swab was emulsified in 2 to 4 cc. of physiological saline and a loopful added to 15 cc. of melted agar cooled to 48°C. from which a blood agar plate was poured. After 24 hours' incubation if hemolytic colonies were seen on the plates at least 2 colonies from both the surface and pour plates were picked to another blood agar plate. In the pour plates a search was made for the minute hemolytic streptococci described by Long and Bliss[5] and the plates were kept under observation for 4 days before they were discarded. If the transfers from individual hemolytic colonies proved to be streptococci they were transferred to rabbit blood broth which was examined the next day for hemolysis. A pour blood plate was made from these blood broth tubes. Only the cultures which produced hemolysis in the blood broth and showed typical beta type hemolysis in blood agar pour plates were classified as hemolytic streptococci. Several alpha prime organisms (Brown) were isolated and are under investigation for their possible pathogenic significance, but they are not included in this report.

Antistreptolysin determinations were carried out according to the method of Todd.[6] The "units" of antistreptolysin titer reported here represent the reciprocal of the fraction of a cubic centimeter of the serum required to neutralize a standard amount of the hemotoxin liberated by the Aronson strain of hemolytic streptococcus. Thus a neutralizing dose of 0.01 cc. of serum is equivalent to 100 units of antistreptolysin. Todd considers this value the upper limit of normal in the serums of healthy individuals without a preceding hemolytic streptococcus infection.

* All the culture medium used was prepared in New York and sent South. It was beef muscle infusion medium made up with neopeptone and adjusted to pH 7.6.

[5] Long, P. H., and Bliss, E. A., *J. Exp. Med.*, 1934, **60**, 619.

[6] Todd, E. W., *J. Exp. Med.*, 1932, **55**, 267, and *Brit. J. Exp. Path.*, 1932, **13**, 248.

Skin tests with the streptococcus nucleoprotein were done in New Orleans, Dallas, and Galveston during the months of May and June, 1933, on a group of patients and on healthy children. The nucleoprotein solution used was supplied to us by Dr. Heidelberger and was derived from *Streptococcus hemolyticus,* strain C203. It was the "K" fraction precipitated by acetic acid and extractable (after removal of less alkaline extracts) between pH 11 and 13.3. The dilution used for the skin tests, after sterilization by passage through a Berkefeld filter, contained 1 mg. N per 100 cc. One-tenth cubic centimeter of this solution, or 0.001 mg. of N, was injected into the skin of the forearm. Readings were made in 24 hours, the presence and extent of erythema, and induration being noted. A reaction, 2 cm. or greater in diameter, associated with palpable induration, was considered a positive test.

Results. Throat Cultures: One hundred and seven throat cultures were taken from a total of 79 individuals in the period January 2nd to February 12, 1934. Studies in 11 patients who had had streptococcus disease are not included in this report. The remaining 68 individuals are divided into groups consisting of acute glomerulonephritis, acute pharyngitis, controls with a history of recent sore throat and controls without a history of recent sore throat. The hemolytic streptococcus was recovered from the throats of 29 of the members of the total group and the minute hemolytic streptococcus from 10. It will be seen from the table that hemolytic streptococci (beta type) were obtained in 8 of 10 cases of acute nephritis, in 9 of 13 cases of acute pharyngitis, in 3 of 12 controls with history of recent sore throat and in 9 of 33 controls without a history of recent sore throat. In addition the minute form of the hemolytic streptococcus described by Long and Bliss was isolated from 10 throats as indicated in the table.

It would appear from this limited series of throat cultures, taken

TABLE I.

Occurrence of Hemolytic Streptococci in the Throats of Control Individuals and Patients with Acute Glomerulonephritis or Acute Pharyngitis in New Orleans.

Disease	No. of patients	No. showing hem. strep. (beta) in throat cult.	No. showing minute hem. strep. (Long and Bliss) in throat cult.
Acute glomerulonephritis	10	8	4
Acute pharyngitis (includes 4 cases of scarlet fever)	13	9	1
Controls *with* history of recent sore throat	12	3	0
Controls *without* history of recent sore throat	33	9	5

during the height of the winter season in New Orleans, that the hemolytic streptococcus was quite readily recoverable from the throats of individuals suffering from acute nephritis and acute pharyngitis. A certain number of normal individuals were also found to carry the hemolytic streptococcus in their throats.

Antistreptolysin titrations were carried out on the serums of 12 patients with acute glomerulonephritis, 55 control patients without recent sore throat, and 37 unselected medical students. Single determinations were made in the control groups. Serial tests were performed in the patients with acute nephritis. The maximum titers in the latter group and the single determinations in the control groups are shown in Table II.

TABLE II.

Maximum antistreptolysin titer in patients with acute glomerulonephritis	Antistreptolysin titer in control patients without recent sore throat				Antistreptolysin titer in 37 unselected medical students, 21 of whom had had mild upper respiratory infect.		
55	10	25	55	71	16	62	111
100	12	25	55	71	16	66	125
111	14	25	55	83	20	71	125
144	16	33	62	100	20	71	125
166	16	33	62	100	25	83	144
200	16	33	62	100	33	83	166
200	16	33	62	100	33	83	166
200	20	33	62	100	50	83	200
500	25	33	62	111	50	83	
						83	
500	25	33	71	125	55	100	
500	25	50	71	125	55	100	
555	25	50	71	166†	55	100	
	25	50	71	250‡	62	100	
	25	50	71		62	100	

† This patient may have had acute glomerulonephritis.
‡ This patient had aestivo-autumnal malaria.

It is evident from these limited observations that the antistreptolysin titer of the control patients without a recent sore throat is similar to that reported by Todd[6] for normal individuals in England and by Coburn and Pauli[7] for subjects without recent hemolytic streptococcus disease in New York City. The higher titers of the medical students in New Orleans are probably explained by the increased exposure of this group to respiratory infection. Twenty-one of the 37 students had recently had an upper respiratory infection.

Nine of the 12 patients with acute glomerulonephritis show serum

7 Coburn, A. F., and Pauli, R. H., *J. Exp. Med.*, 1932, **56**, 651.

antistreptolysin values which are above the normal value. These figures are indicative of recent infection with hemolytic streptococcus. Similar high titers of this antibody were found in the serums of 20 out of 22 patients with acute glomerulonephritis in New York.[8]

Skin Reactivity to Streptococcus Nucleoprotein in New Orleans, Dallas and Galveston: A total of 152 individuals were tested intracutaneously with 0.1 cc. of a solution of nucleoprotein of the hemolytic streptococcus containing 0.001 mg. N. Fifty-five responded in 24 hours with an area of erythema at least 2 cm. in diameter and palpable induration. The subjects tested were 85 adult patients both negroes and whites with a variety of medical or surgical conditions, and 67 children from 3 to 12 years of age. None of these individuals was known to have had hemolytic streptococcus disease. The number of positive reactions to the nucleoprotein of the hemolytic streptococcus in residents of these southern cities was approximately the same as that found by us in New York when we tested a group of patients on the medical wards of the Presbyterian Hospital with the same lot of nucleoprotein just before our study in the South. Coburn[9] had previously found a similar percentage of positive skin reactions with this streptococcus product in a comparable control group. Schroeder[10] obtained practically identical results in a large group of individuals living at or near the equator (Bangkok, Singapore, Colombo) whom he tested intradermally with the same sample of nucleoprotein in the fall of 1933.

The results of skin tests with nucleoprotein of the hemolytic streptococcus seem to indicate that there is the same degree of skin reactivity to this substance in the South as in New York. The significance of a positive test is open to argument but it suggests acquired sensitization to the hemolytic streptococcus since skin reactions are known to increase in frequency and severity in hemolytic streptococcus disease (Coburn).

Conclusions. This limited bacteriological and immunological search for evidence of the presence or absence of the hemolytic streptococcus in a relatively southern city, where one type of probable hemolytic streptococcus disease (glomerulonephritis) is present in "normal" amount and other types are decreased has shown that

[8] Seegal, D., and Lyttle, D., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 211.

[9] Coburn, A. F., *The Factor of Infection in the Rheumatic State*, Williams & Wilkins Company, Baltimore, 1931.

[10] Schroeder, H. A., unpublished data.

the hemolytic streptococcus can be cultivated from the throats of a majority of individuals with acute nephritis. It has been possible to demonstrate normal carriers for the hemolytic streptococcus. Furthermore, antibody active against the soluble streptolysin (hemotoxin) of *S. hemolyticus* was increased in cases of acute glomerulonephritis. There also was the same degree of skin reactivity to the nucleoprotein of the hemolytic streptococcus in the South as in New York. These bacteriological and immunological findings therefore substantiate the clinical data already reported and give still further evidence that acute glomerulonephritis in the South as in the North is usually the result of a hemolytic streptococcus infection.

No opportunity was available to evaluate the relative frequency of the hemolytic streptococcus in the throats of the population at large in New Orleans as compared with New York nor to determine its seasonal incidence. Information on both these subjects would be interesting and possibly helpful in solving the basic problem which still remains, namely the reason for the "normal" incidence of one type of hemolytic streptococcus disease (acute glomerulonephritis) in the presence of a decreased incidence of other types of hemolytic streptococcus disease (scarlet fever, rheumatic fever) in the South. All strains isolated in New Orleans were brought back to New York and are being compared culturally and immunologically with a series of hemolytic streptococci isolated from the throats of individuals in New York City.

8012 C

Vitamin C and Diphtheria Toxin.

CHARLES K. GREENWALD AND E. HARDE.

From the Bureau of Laboratories, Department of Health, New York City.

In previous work,[1] a relation between Vitamin C and diphtheria toxin was found. Freshly prepared highly labile toxin was used.

In the present work these findings were confirmed and extended using a standardized, stable toxin. In relation to this toxin, we have examined 2 different actions, (a) the effect on guinea pigs of preliminary feeding of excess Vitamin C (cevitamic acid) and of

[1] Harde, E., *C. R. de l'Acad. des Sc.*, 1934, **199**, 618; Harde, E., and Phillipe, M., *C. R. de l'Acad. des Sc.*, 1934, **199**, 738.

treatment with the vitamin,* and (b) the action *in vitro* of cevitamic acid on the toxin, on antitoxin and on mixtures of toxin and antitoxin.

A. Effect on guinea pigs of preliminary feeding of excess vitamin C (cevitamic acid) and of treatment with the vitamin. Four guinea pigs were fed excess spinach or cabbage in addition to the stock diet of hay, oats and carrots for 10 days. They were then injected with 1 M.L.D. of toxin and kept on the same diet. No increased resistance was shown as compared with controls fed the stock diet. In a second experiment, 5 guinea pigs were given 10 mg. of pure cevitamic acid in ½ to 1 cc. salt solution by mouth daily for 3 days. Three of these animals were also injected daily, intramuscularly or subcutaneously,† with 10 mg. of the cevitamic acid in a similar solution. These animals were then injected with 1 M.L.D. of standardized toxin, and the vitamin C treatment continued daily. In comparison with the control animals injected with 1 M.L.D. at the same time, no increased resistance was shown, all the animals dying in 4 to 5 days.

These results differed from those obtained in previous work using a freshly prepared toxin, in which 3 out of 6 animals survived, and 5 controls died. A freshly prepared toxin is very unstable, losing rapidly a certain part of its toxicity, and it may also be more sensitive to vitamin C.

Animals injected with toxin may be extremely sensitive to the shock of handling. Occasionally during the feeding one of these animals died suddenly. There is always the possibility of some of the liquid going into the bronchi. Autopsies have shown this in a number of cases. We have also noticed at autopsy that subcutaneous or intramuscular injections of vitamin C were not always completely absorbed.

In another series of experiments, therefore, the animals were handled with great care so that there was very little shock, only the vitamin C solution being fed and injections avoided. A salt solution of 10-20 mg. of cevitamic acid was given to each of 4 guinea pigs; after the first or second day, each was injected with 1 M.L.D. of standardized toxin, and the feeding continued daily. The controls died on the 4th day. All the treated animals lost weight and showed indura-

* We thank the Hoffman LaRoche Company for the Redoxon-Cevitamic Acid they kindly supplied us.

† These solutions were always rendered neutral to litmus using a sodium hydroxide solution and sterilizing by bringing to a boil. To some animals carotene was also given by mouth without any effect being noted.

tion, one died on the 5th day and 1 on the 6th day. To the 2 others, vitamin C feeding was continued until the 8th day. These animals survived 20 and 22 days, dying of pneumonia. A second series gave similar results. One animal died on the 3rd day with lung lesions, one on the 9th day, and the remaining 2 survived. The 4 controls died in from 3 to 5 days.

B. Action *in vitro* of cevitamic acid on toxin, on diphtheria antitoxin and on mixtures of toxin and antitoxins. To 1 or 2 M.L.D.'s of toxin in 1 cc. of salt solution was added 1 cc. of a neutral solution of cevitamic acid, 10 mg. per M.L.D. This was slowly mixed, care being taken not to bubble air through it. Four guinea pigs injected with these solutions showed no loss of weight and no local symptoms, while the controls died in 4½ days. This experiment was repeated with similar results. With 5 M.L.D.'s using 10 mg. of the vitamin per M.L.D., 2 guinea pigs survived and 2 died ten days after injection. With a toxin solution containing 10 M.L.D.'s and treated with the same amount of vitamin C and injected, 2 animals survived, while 2 animals injected with 10 M.L.D.'s to which 60 mg. of vitamin C had been added died after 4 days, and one guinea pig receiving 20 M.L.D.'s and 100 mg. of vitamin C died in 48 hours.

We are now testing whether prolonged contact or increased amounts of cevitamic acid are necessary for action on the toxin.

In the earlier experiments a partial immunity was found in animals that had survived these injections when tested with 1½ M.L.D.'s. In this work the surviving animals were injected with 2 M.L.D.'s 5 to 6 weeks after the first injection. They all died in 2 to 4 days, suggesting that there had been little if any immunity produced by the first injection.

If vitamin C is to be used clinically as an adjunct to antitoxin in the treatment of diphtheria, it is necessary to test its action on the antitoxin.

To a standard antitoxin was added 10 mg. per unit of cevitamic acid, dissolved in a neutral salt solution. This was thoroughly mixed but not aerated and allowed to remain for 24 hours at 4°C. protected from the light. Two guinea pigs were injected with this mixture to which was added ½ L+ of toxin, 2 with one L+, and 2 with 1½ L+. For controls a freshly prepared standard unit of antitoxin was used with similar amounts of toxin. The only difference in resistance between these groups was shown in the one L+ dose. The guinea pigs receiving the solution containing the vitamin C lived 2 days longer than the controls, which died between the 3rd

and 4th days. No deleterious action of Vitamin C on the antitoxin was shown. Experiments were then done to note if the vitamin had any effect on toxin antitoxin mixtures. One unit of antitoxin, one L+ of toxin, and 10 mg. of cevitamic acid in salt solution were made up to 4 cc. with a salt solution, carefully mixed without aeration and kept at room temperature protected from the light for one hour. Controls without the cevitamic acid were kept under the same conditions. Three guinea pigs were injected in each series. The controls died within 4 days while the animals injected with the cevitamic acid mixture showed no symptoms and survived.

A final experiment was made with unneutralized cevitamic acid. One hundred and fifty mg. of the vitamin was dissolved in one cc. of standardized toxin, 500 M.L.D.'s per cc. The pH was between 4 and 4.4. The mixture was kept for 18 hours at room temperature. The guinea pigs were injected with one, 1¼ and 2 M.L.D.'s of the treated mixture. They showed no effects of the injection while the controls died. Here the vitamin was neither heated nor neutralized and was only added in the proportion of one mg. to 3½ M.L.D.'s. Further experiments are necessary to determine what factors are responsible for this detoxification.

Summary. Vitamin C under certain conditions increased the resistance of guinea pigs to injection of 1 M.L.D. of a standardized diphtheria toxin. Injections of mixtures of toxin and vitamin C if the solutions had been in contact for 1 hour at room temperature before injection were less toxic. The guinea pigs that had survived these injections 5 to 7 weeks showed but slight if any immunity when tested by the injection of 2 M.L.D.'s of the toxin. Vitamin C caused no destruction of the antitoxic properties of antitoxin or of the slightly toxic toxin antitoxin mixture. Only clinical work can determine whether the increased resistance to diphtheria toxin shown by guinea pigs treated with vitamin C can be applied to human beings. While we were engaged in these experiments Dr. C. G. King told us that he and Dr. Bessey[2] were working in the same field and had also found a relationship between vitamin C and diphtheria toxin.

We wish to thank Mr. Andrew Mackey for his aid in carrying out the experiments.

[2] Bessey, O. A., and King, C. G., *J. Nutrition.* In press.

8013 P

Relation of Vitamin B (B_1) Intake to Neurological Changes in the Alcohol Addict.

NORMAN JOLLIFFE AND PHILIP M. JOFFE.

From the Department of Medicine, New York University College of Medicine, and the Psychiatric Medical Service of the Third (New York University) Medical Division, Bellevue Hospital, New York.

Cowgill[1] found that the Vitamin B requirement of man could be predicted with reasonable accuracy by the following equation:

$$\frac{\text{Vitamin B mg. Eq.}}{\text{Calories}} = 0.0000284 \text{ Weight in gm.}$$

From the nature of the equation the expression $\frac{\text{Vitamin B mg. Eq.}}{\text{Calories}}$ (hereafter referred to as the Vit/Cal ratio) may be used as an indicator of the adequacy or inadequacy of the diet of any individual in respect to this vitamin.

Peripheral neuritis in the alcohol addict has been attributed to vitamin B deficiency.[2] To obtain more evidence on the deficiency theory we have calculated the Vit/Cal ratio in the diets of 24 alcohol addicts. Only those subjects who gave reliable histories, which we checked by repeated questioning and usually verified through friends or relatives, were used. Average portion weights[3] and Cowgill's[1] tables were used as a basis for calculation of the caloric and vitamin B values of the various foods. Two Vit/Cal ratios were determined, one omitting and the other including the calories derived from alcohol. Each ratio was compared to the predicted requirement.

Neurological observations made shortly after the admission of each patient are compared to the calculated Vit/Cal ratios. When the alcohol calories are omitted, 17 of the 24 subjects appear to have had an adequate vitamin B supply. However, when the alcohol calories are included, the Vit/Cal ratio in 20 of the 24 subjects falls below Cowgill's predicted value, indicating an inadequate vitamin intake. Each subject who showed a peripheral neuritis had an inadequate Vit/Cal ratio for at least 22 days, whereas not a single

1 Cowgill, G. R., *The Vitamin B Requirement of Man*, Yale University Press, 1934.

2 See Minot, G. R., Strauss, M. B., and Cobb, S., *New England J. Med.*, 1933, **208**, 1224, for literature.

3 Unpublished compilation prepared chiefly from Basic Quantity Food Tables, Department of Public Charities, City of New York, 1917.

subject having an adequate Vit/Cal ratio showed significant neurological changes even though alcohol had been used over a long period of time. The consumption of large amounts of spirituous liquor for a period as long as 18 days, with a low Vit/Cal ratio, leads to no abnormal neurological signs except tremor and hyperreflexia. It appears that the alcohol addict, to maintain an adequate Vit/Cal ratio, should supplement an otherwise adequate diet with approximately 250 mg. equivalent (50 Sherman Units) of vitamin B for each ounce of liquor consumed. This amount of vitamin B is contained in about 1.5 gm. of Vegex (autolyzed brewer's yeast).

Six subjects who had cord changes, and 5 with brain changes, had an inadequate Vit/Cal ratio for at least 2 months. It is possible that these changes in the central nervous system are due to vitamin B deficiency, the cord and brain being merely less susceptible to the lack of vitamin B than the peripheral nerves.

Cowgill uses the caloric intake to express for practical purposes the energy exchange of the individual. The practically complete utilization of moderate amounts of alcohol is well known. However, the energy exchange calculated from the caloric intake may not represent the true energy exchange when large quantities of alcohol are consumed, since a considerable portion may be excreted unchanged through the intestinal tract (vomiting and diarrhea), the lungs and kidneys.

Conclusions. The Cowgill formula predicting the vitamin B requirement of man has been applied to the diets of alcohol addicts in order to estimate the adequacy of the vitamin B intake. Sixteen subjects showing peripheral neuritis had for at least 22 days an inadequate Vit/Cal ratio. Four subjects who consumed large amounts of liquor, but with an adequate Vit/Cal ratio, showed no abnormal neurological signs. Four subjects having an inadequate ratio for 18 days or less showed no significant neurological changes. These findings support the theory that peripheral neuritis in the alcohol addict is the result of vitamin B deficiency.

8014 P

Observations on Excretion of Vitamin C in Some Vascular Diseases.*

PHILIP FINKLE. (Introduced by Geo. Baehr.)

From the Division of Laboratories and from the Medical Service of Dr. George Baehr, the Mount Sinai Hospital, New York.

The urinary excretion of vitamin C by normal individuals has been studied by Harris, Ray, and Ward, and was found to average about 0.03 mg. per cubic centimeter of urine for 24 hours.

What rôle, if any, vitamin C plays in the evolution of pathological states associated with vascular damage and hemorrhagic tendencies other than in scurvy is still unknown.

Studies were therefore undertaken upon the excretion of vitamin C in the urine in certain pathological conditions. The rate of vitamin C excretion following the intravenous administration of 100 mg. of cevitamic acid (vitamin C) was also studied. Here are presented the results of these studies upon cases of various types of purpura, metrorrhagias, and 2 cases of acute lupus erythematosus, all of which conditions are associated with tendency to hemorrhage and with a greater or lesser degree of vascular damage.

In the titration of cevitamic acid 2:6-dichlorophenolindophenol was used as the indicator.

TABLE I.
Excretion in Urine of Cevitamic Acid (Vitamin C) Before and Following Intravenous Injection of 100 mg. Cevitamic Acid.

Name	Diagnosis	Aver. Daily Mg. Vitamin C per cc. Urine	Mg. Vitamin C per cc. urine 2 to 3 hr. following injection	Mg. Vitamin C per cc. urine 4 to 6 hr. following injection
E.M.	Normal Control	.025	.195	.09
M.F.	"	.04	.25	.08
D.S.	"	.025	.15	.05
M.L.	"	.03	.17	.04
E.G.	Purpura Hemorrhagia	.006	.007	.007
R.LaC.	"	.02	.012	.02
I.E.	"	.006	.005	.008
A.K.	"	.019	.011	.019
S.C.	"	.016	.02	.02
E.Z.	Metrorrhagia	.01	.02	.007
M.L.	"	.009	.007	.006
F.B.	"	.017	.017	.028
R.B.	"	.017	.015	.016
H.B.	Acute Lupus Erythematosus	.01	.012	.011
M.G.	"	.015	.021	.016

* Aided by a grant from Mr. Nelson I. Asiel.

In comparing the values for vitamin C excretion in normal urines with those found in the pathological conditions studied, the writer found what appears to be a significant difference between them. In the latter cases the excretion was considerably lower than normal, as much as 80% in some instances. This marked diminution in vitamin C excretion was observed in the cases of purpura and metrorrhagias, as well as in the cases of lupus erythematosus.

Following the intravenous administration of cevitamic acid, the excretion in normals rose to about 5 to 6 times the values found before the intravenous injections. This rise occurred in about 2 hours following injection, and came down to the normal level in about 4 to 6 hours.

The pathological cases studied showed a striking difference from the normal in their excretion curves, following intravenous administration of cevitamic acid. There was practically no change in urinary excretion of vitamin C. Not only was there no rise in excretion following injection, but the values still remained considerably lower than the normal.

One of the patients with acute lupus erythematosus was given intravenously 200 mg. of cevitamic acid daily for 6 days. She then received about 500 cc. of orange juice daily for one month. While the excretion level rose to normal after this prolonged administration of fairly large quantities of vitamin C, there was a rise only to double the output following intravenous administration of 100 mg. of cevitamic acid. This is a considerably smaller rise than occurred in normals.

A normal control was studied, after he had refrained for 5 days from taking orange juice or vitamin C in other forms. The excretion level was still normal, rising to 5 times the level following intravenous injection.

At present it is not possible to appraise the significance of these results in the diseases studied.

8015 C

Detection of a Healthy Carrier of Virus of Poliomyelitis Without History of Contact.*

S. D. KRAMER.

From the Laboratories of the Infantile Paralysis Commission, Long Island College of Medicine and the Bureau of Laboratories, Department of Health, New York City.

We present evidence that healthy carriers of the virus of poliomyelitis without history of contact and in the absence of an outbreak of the disease do exist and to discuss briefly the significance of this finding. The problem was undertaken in an effort to explain the widespread immunity in the normal population to this rare disease. A healthy carrier rate for diphtheria has been established, sufficient in extent to account for the widespread immunity to that disease. As extensive an immunity in the normal population is found in the case of poliomyelitis, a disease one hundred times as rare as diphtheria. It has seemed reasonable to assume that the mechanism involved in such widespread immunity was the same in the two diseases. Specific experimental evidence of the existence of such healthy carriers without history of contact has thus far been lacking.

The virus was recovered from the tonsils and adenoids of recovered cases,[1] and from the nasal washings of contacts and of mild illnesses occurring in close proximity to cases.[2-5] It has also been shown[6] that individuals exposed to the virus of poliomyelitis may develop immunity to the disease without evidence of illness. However individual contacts with cases fail to explain the widespread immunity that is found in the normal population to this disease.

Tonsils and adenoids were selected as the source of the virus, because it has been shown[1] that these portions of the mucous membrane can harbor the virus and because they were readily obtainable in large numbers.

* This work is supported by a grant from the Rockefeller Foundation, an anonymous gift, and by private contributions.

1 Flexner, S., and Amos, H. L., *J. Exp. Med.*, 1919, **29**, 379.

2 Flexner, S., Clark, P. F., and Fraser, F. R., *J. Am. Med. Assn.*, 1913, **60**, 201.

3 Kling, Pettersson, and Wernstedt, *15th Internat. Congr. Hyg. and Demography*, Washington, 1912, p. 5.

4 Taylor, Edw., and Amos, H. L., *J. Exp. Med.*, 1917, **26**, 745.

5 Trask, J. D., and Paul, J. R., *J. Exp. Med.*, 1933, **58**, 531.

6 Kramer, S. D., *J. Am. Med. Assn.*, 1932, **99**, 1048.

Through the coöperation of the nose and throat department of the Long Island College Hospital, the tonsils and adenoids removed in the weekly routine operations were collected within an hour following removal. Each set of tonsils and adenoids was placed in a separate container and identified by name of patient and hospital number, then set in the freezing compartment of a refrigerator and kept there until used; (seldom more than 12 to 24 hours). Part of each of the tonsils and adenoids was removed and the fragments, of 2 to 5 patients, were pooled, ground with sterile sand and made up in 10% suspension. The remainder of each of the tonsils and adenoids was placed in individual bottles containing 50% sterile glycerol, identified by name of patient and lot number of pooled tissue and stored in the ice chest. Part of the pooled suspension (about 5 cc.) was set aside for intranasal instillation. The remainder of the material was filtered through a Seitz pressure filter. This procedure usually yielded 20 to 50 cc. of a slightly red tinged sterile liquid.

Recognizing the difficulties in obtaining a primary "take" from this material, we utilized as many routes of inoculation as possible. Healthy monkeys were selected, bled for preliminary testing for the presence of neutralizing substance, and inoculated: (1) 1 to 2 cc. of the unfiltered material instilled into each nostril. (2) 2 cc. of the filtered material inoculated intracerebrally. (3) The remainder of the filtered material inoculated intraperitoneally. The animals were isolated, temperatures taken and observations of their behavior made daily.

The first animals were inoculated March 1934 and nothing unusual was observed in any of the animals until June 21st when monkey 107, inoculated with Lot 16, pooled from 5 individuals, showed elevated temperature to 105°F. On June 25th the temperature was 106°F. The following day the temperature dropped to 102°F., which was essentially the normal temperature of the animal. The animal was observed for other symptoms and except for a disinclination to climb, nothing unusual was observed. The animal was not used again and was observed for further symptoms. In the course of the next few months, the animal did not climb as well or as quickly as the other animals. A neutralization test October 6th, showed a prolonged incubation period. The control animal was prostrated in 8 days whereas the animal used for testing the serum of monkey 107 came down with a mild illness 16 days after inoculation. At this time the muscles of the right thigh were atrophied with beginning contracture of the leg on the thigh (the

right thigh was 2 cm. less in circumference than the left.) The animal was exsanguinated, quickly sacrificed with chloroform, the cord and brain exposed and sections taken from different levels for histologic study.

Attempts to Identify Source of the Virus. The remainder of the material from Lot 16 was ground separately with sand under sterile precautions in 10 to 50% suspensions (depending upon the amount of material available), centrifuged and inoculated unfiltered, intracerebrally, into each of 5 monkeys on November 22.

On November 27, monkey 234 inoculated with material from a child, V. R. 2 years old, showed an elevation of temperature to 106.2°F. The following day the temperature was 104.2°F. Cistern tap on that day showed 140 cells and a positive Pandy. The animal was sacrificed with chloroform. The brain and cord were moderately injected. No brain abscess was found. Sections were taken from the cord for histologic study, and the remainder was glycerolated. A portion of the cord was ground in 10% suspension and inoculated into 6 animals.

Monkey 371 inoculated on 12/20/34 with the cord suspension from monkey 234 showed an elevation of temperature on the 26th. The animal was sacrificed with chloroform; sections were taken for histologic study and part of the cord ground in 10% suspension and inoculated into 4 animals. The remainder of the cord was glycerolated and stored.

Monkey 391 inoculated 1/11/35 with suspension of cord 371, showed no marked elevation of temperature but on the 21st, definite weakness of the left shoulder was observed. This animal developed a severe diarrhea, which may account for absence of temperature response. Monkeys 234 and 371 appeared ill with the rise in temperature but no definite paralysis. Monkey 391 was the first animal to show paralysis although not extensive. The animal was sacrificed under chloroform anaesthesia. There was an unusual amount of injection of both cord and brain. Sections were taken for histologic study; part of the cord was ground into suspension for further passage and the remainder was placed in 50% sterile glycerine and stored.

Monkey 409 inoculated on January 21, with material from monkey 391 succumbed to frank poliomyelitis February 1, showing involvement of the left shoulder and partial paralysis of both lower extremities. The animal was sacrificed; sections removed for histologic study; part of the cord ground up for further passage and the remainder placed in 50% sterile glycerine and stored. At post-

mortem none of these animals showed any evidence of other illnesses.

Histologic Examination. Monkey 107. The following description is from an H & E stain of a section taken from the lower lumbar and sacral region. There is no meningitis. The pia is moderately hyperplastic. In the gray matter the following changes are observed: (a) A disproportion in the number of neurons in the anterior horns of both sides. (Figs. 1 and 2). (b) In the horns con-

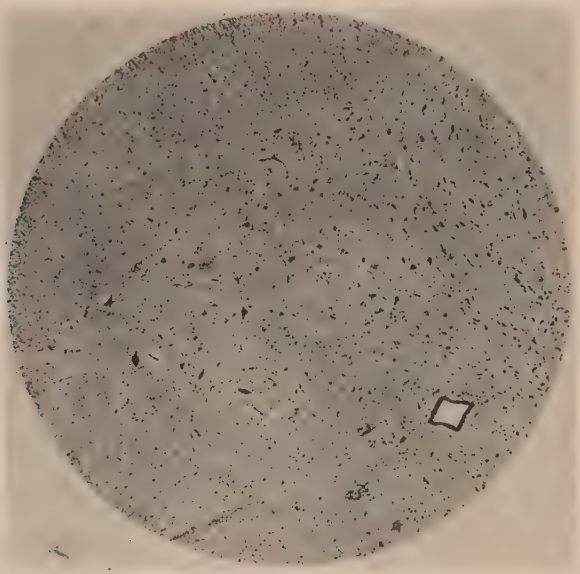


FIG. 1.

Monkey No. 107 H and E. High power of the anterior horn of the atrophied area, showing reduced number of neurons and evidence of degeneration in many of the remaining neurons.

taining the larger number of neurons there is abnormal staining with hemotoxylin. Some of the nuclei are pycnotic and in some cells the nuclei are absent. Some of the neurons show satalitosis and karyolysis. There are cell bodies in various stages of degeneration, almost to the stage of total disappearance. There is a moderate increase in the number of microglia and oligodendroglia cells. (c) In the opposite horn, the same changes occur but more of the neurons have undergone degeneration, to total disappearance. Remnants of cells devoid of nuclei and barely recognizable are seen throughout. The changes are therefore similar but more extreme than those observed in the opposite horn and fewer neurons have survived. (d) There are only moderate generalized infiltrations with lymphocytes,

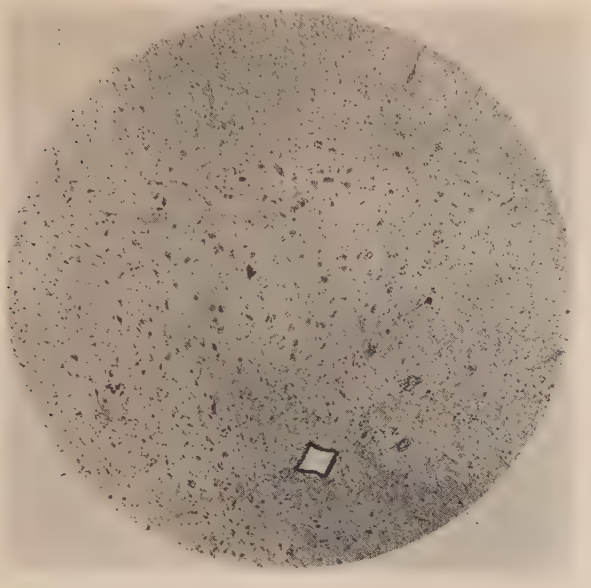


FIG. 2.

Monkey No. 107 H and E. High power of the opposite horn, showing numerous anterior horn neurons, many of which are in various stages of degeneration.

mononuclears and oligodendroglia cells. These changes are compatible with poliomyelitis.

Monkey 234. There is definite infiltration of the gray matter with polymorphonuclear leucocytes and lymphocytes in moderate numbers. Definite neuronophagia. Many of the neurons show chromatolysis, cytolysis and satalitosis. The entire gray matter appears oedematous. There is an increase in microglia and oligodendroglia cells. The pia is hyperplastic and there is a subarachnoid exudation of albuminous fluid and moderate numbers of polymorphonuclear leucocytes and lymphocytes, chiefly polys. There are no marked vascular changes.

Monkey 371. The pia shows local leucocytosis chiefly with polymorphonuclear leucocytes. There is a cellular increase throughout the gray matter chiefly with microglia and oligodendroglia cells. There are some polymorphonuclear leucocytes. The ganglion cells show moderate degenerative changes; chromatolysis, eccentric arrangement of the nucleii, cytolysis, satalitosis and neuronophagia. There are no extensive vascular changes.

Monkey 391. The pia shows intensive inflammatory reaction. There is extensive infiltration with lymphocytes, plasma cells and polymorphonuclear leucocytes. The gray matter shows extensive

infiltration. There is a marked microglial reaction. The ganglion cells show extensive cloudy swelling and neuronophagia. Many of these cells have been replaced with mononuclears. The blood vessels show extensive inflammatory reaction. There is infiltration with lymphocytes and plasma cells. (Fig. 3).

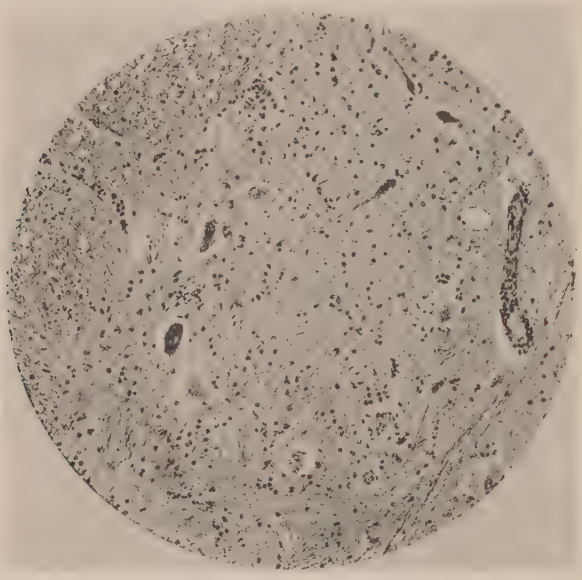


FIG. 3.

Monkey No. 391 H and E. Illustrating generalized infiltration, perivascular infiltration, and various stages of degeneration of anterior horn neurons.

Monkey 409. Presents essentially the same picture as Monkey 391. The last 4 animals give histologic evidence compatible with poliomyelitis, although the changes noted in the latter 2 are more progressive and definitely identified as poliomyelitis.

V. R., the 2-year-old child, whose tonsils and adenoids had been inoculated into Monkey 234, was investigated. She was a healthy girl whose tonsils had been ordered removed by the family physician because of frequent upper respiratory infections. The hospital record diagnosis was hypertrophied tonsils and adenoids. There was no history of contact with a case of poliomyelitis. 1934 was the year of lowest incidence on record for Brooklyn.

The finding of a healthy carrier, without any history of contact, in the normal population has, in our opinion, considerable significance. It perhaps points the way toward establishing the epidemiology of poliomyelitis on as firm a basis as the better known and more common disease, diphtheria.

The assumption of the existence of healthy carriers to explain the widespread immunity in the normal population to so rare a disease has been emphasized repeatedly. The finding of such a carrier strongly suggests that the mechanism involved in immunizing the general population is similar to that accepted for diphtheria. In the latter disease, to which about nine-tenths of normal urban adults are immune, it has been calculated that the number of healthy carriers of the diphtheria organism (variously estimated between ½ and 2%) is sufficient to saturate the population over a period of 20 years.

An equally large incidence of immunity is found in the adult urban population to poliomyelitis. In view of the relative rareness of poliomyelitis, offering fewer opportunities for contact with cases, it is more difficult to explain such extensive immunity than in the case of diphtheria. The presence of healthy carriers of the virus of poliomyelitis explains adequately the widespread immunity to this disease.

The frequency of such carriers cannot be stated. The difficulties involved in establishing a human strain of virus in the monkey are well known. It frequently requires 3, 4 or more inoculations to effect a primary "take" in the monkey with human material. In view of these difficulties, the single finding here reported, takes on added numerical significance. The determination of the actual carrier rate must take these factors into consideration and the final figure will probably be greater than the positive finding of the one in 156 or .64% of the normal individuals included in this report, although even this figure would be sufficient to fulfill the mathematical requirements to account for the widespread distribution of the virus, as well as immunity, in an urban population.

Summary. 1. Tonsil and adenoid tissue obtained from a healthy child 2 years old, without any history of contact, produced the experimental disease when inoculated into 2 animals. 2. One of the animals, Monkey 107, recovered with atrophy and contractures involving the right lower extremity. 3. The other animal sacrificed in the acute stages of the disease showed histologic evidence of poliomyelitis and the material from the cord of this monkey has produced the frank recognizable disease with paralysis in the third and fourth passages (Monkeys 391 and 409).

This investigation is being continued on a more extensive scale with the collaboration of Dr. M. Schaeffer of the Department of Bacteriology, New York University, with material obtained from the Jewish Hospital of Brooklyn. It is hoped that by including

other sections of the community, a more exact estimate of the carrier rate and the geographic and seasonal distributions can be made.

8016 C

Immunological Relationships of Strains of Filtrable Virus Recovered from Cases of Human Influenza.

THOMAS FRANCIS, JR. (Introduced by Rufus Cole.)

From the Hospital of the Rockefeller Institute, New York.

Smith, Andrewes and Laidlaw[1] isolated a filtrable virus from the nasopharyngeal washings of influenza patients following the inoculation of these materials into ferrets. Two strains of virus isolated by them in England during successive winters were found to be immunologically identical.[2] These same workers reported that the virus of swine influenza isolated by Shope[3] was antigenically related to the human strains.

During the Autumn of 1934, at the Hospital of the Rockefeller Institute, we were successful in infecting ferrets and mice with strains of a filtrable virus obtained from the sputum of cases of epidemic influenza in Puerto Rico.[4] These 2 strains have been called P. R. 5 and P. R. 8. Additional strains of virus have been isolated from cases of influenza in New York and Philadelphia. During the course of these experiments, Andrewes, Laidlaw and Smith[2] reported independently that they had successfully infected mice with the viruses of both swine and human influenza. They also reported that the serum of a hyperimmune horse, or of hyperimmune ferrets, neutralized the infectivity of the respective strains of virus.

The infection in mice, following the intranasal inoculation of the virus is characterized by the development of pulmonary lesions, but death of the animals is somewhat irregular. The serum of ferrets recovered from infection, when mixed with suspensions of the homologous strain of virus and instilled into the nasal passages of mice, has been found to prevent the development of these pulmonary lesions. The serum of normal ferrets, however, has no neutralizing

1 Smith, W., Andrewes, C. H., and Laidlaw, P. P., *Lancet*, 1933, **2**, 66.
2 Andrewes, C. H., Laidlaw, P. P., and Smith, W., *Lancet*, 1934, **2**, 859.
3 Shope, R. E., *J. Exp. Med.*, 1931, **54**, 373.
4 Francis, T., Jr., *Science*, 1934, **80**, 457.

TABLE I.
Protection of Mice Against Influenza Virus.

Strain of Virus		Severity of Pulmonary Lesions									
		P.R.8 Strain					Phila. Strain				
Mouse No.		1	2	3	4	5	1	2	3	4	5
Serum:											
Ferret P.R.8	1:2	0	0	0	0	0	±	0	0	0	0
	1:10	0	0	0	0	0	0	0	+	0	0
Ferret Phila.	1:2	0	0	0	0	0	0	0	0	0	0
	1:10	0	0	0	0	0	0	0	0	0	0
Ferret P.R.5	1:2	0	0	0	0	0					
Ferret Normal	1:2	++++	+++	++++	+++	++++	++++	+++	++++	++++	++++
Immune Horse*	1:2	0	0	0	0	0	0	0	±	0	0
	1:10	0	0	0	0	0	0	0	0	0	0
Normal Horse	1:2	++++	++++	++	+++	++++	++++	++++	+++	++++	+++
Anti-Swine Influenza (Shope)	1:2	+++	++++	+++	+++	++++					

0 = No pulmonary involvement.
± to ++++ = Increasing degrees of pulmonary involvement.
* This serum was obtained through the courtesy of Dr. Andrewes, of the National Institute for Medical Research, London, England.

effect. It is possible, therefore, to measure in mice the neutralizing capacity of the serum of a recovered ferret against homologous and heterologous strains of virus.

The sera available were those of ferrets recovered from infection with one or another of 3 strains of human influenza virus: P. R. 5 strain, P. R. 8 strain, or Philadelphia strain; the serum of a horse immunized by Andrewes, Laidlaw and Smith against the British (W.S.) strain of influenza virus; and the serum, obtained from Dr. Shope, of swine convalescent from infection with Shope's swine influenza virus.

The tests are performed as follows: Weighed amounts of infected mouse lung, as the source of virus, are ground with physiological salt solution to form a 10% suspension. After centrifugation of the suspension for 15 minutes, equal portions of the virus suspension and serum are mixed and incubated at 37°C. for 30 minutes. Each of 5 mice, lightly anesthetized with ether, is then inoculated intranasally with 0.03 cc. of the mixture. On the 5th or 6th day after inoculation, at which time the control mice are dying, all other mice are killed and their lungs removed. The effect of sera of unknown potency upon the infectivity of the virus is measured by comparing the severity of the pulmonary lesions in mice receiving mixtures of those sera and virus, with the lesions in control mice receiving virus and normal ferret or horse serum.

The results of a typical protective experiment are presented in Table I.

It was found that the serum of ferrets recovered from infection with the Puerto Rico and Philadelphia strains reciprocally neutralized both strains of virus (P. R. 8 and Philadelphia), whereas normal ferret serum had no effect. Immune horse serum, prepared by Andrewes and his coworkers against the British strain of virus, also neutralized completely these 2 strains of virus. Serum of swine convalescent from infection with the virus of swine influenza, and known to neutralize swine influenza virus failed to neutralize strains of human influenza virus. These results indicate that the strains of virus recovered from cases of human influenza in Puerto Rico, Philadelphia and England are immunologically identical, whereas the virus of swine influenza differs serologically.

Furthermore, it was found that the serum of human individuals in New York, taken during convalescence from influenza, gave marked protection to mice against the Puerto Rico strain of virus, while the serum of the same individuals taken during the acute stage of the disease did not.

It appears, therefore, that the virus obtained from human influenza is a distinct entity and is etiologically related to the human disease.

8017 P

A Method for Titrating the Protective Action of Antimeningococcal Serum.

GEOFFREY RAKE.

From the Laboratories of The Rockefeller Institute for Medical Research, New York City.

Miller[1] has described a method for the production of experimental meningococcal infection in mice. It consisted in brief of the use of a 6% mucin suspension buffered at 7.4 as a medium in which the organisms were suspended prior to intraperitoneal inoculation. More recently Miller has modified the technique of preparing the mucin.[2] A 5% suspension is now prepared, it is sterilized in the autoclave at 10-15 lb. pressure for 15 minutes, sterile dextrose solution is then added to a final concentration of 1%, and the pH adjusted to pH 7.4 with sterile buffer solution.

Using such a mucin suspension, the intraperitoneal virulence of meningococcus strains can be titrated,[1, 3] and consistent results will be obtained when pure breeds of susceptible mice are employed. It has been found, in accord with Miller's work, that freshly isolated strains may kill when the cultures are diluted as far as 10^{-8}, that is approximately 20 organisms. A brief report has been made elsewhere[3] on the application of this experimental meningococcal infection to the test of sera for their protective activity. That report dealt chiefly with the content of protective antibodies in the serum of carriers of the meningococcus and in the serum of normal individuals. The high protective value of some antimeningococcal sera was demonstrated but no titration was carried out.

In testing the intraperitoneal virulence of strains, 14 to 18-hour cultures on 10% rabbit's blood pneumococcus agar plates are washed off with normal saline and the suspension is diluted in saline and adjusted with a Gates turbidometer to the standard of 2,000,000,000 organisms per cc. Serial dilutions 1:10 are made in mucin and

[1] Miller, C. P., *Science*, 1933, **78**, 340.

[2] Miller, C. P., personal communication.

[3] Rake, G., *J. Exp. Med.*, 1935, **61**, 545.

range between 10^{-1} (200,000,000 organisms) and 10^{-8} (20 organisms). Inoculations are made intraperitoneally into selected susceptible strains of Rockefeller Institute mice, into Swiss and into white-faced breeds.[4, 5] Most mice are dead by the end of the 2nd day. Deaths from the specific infection are very rare after the 4th day.

The intraperitoneal virulence of a strain can be maintained by passage from mouse to mouse and may even increase ten or a hundredfold with such passage.

In testing a serum for protective antibodies, ½ cc. of the serum diluted 2:5 in normal saline is inoculated intraperitoneally half to one hour before the intraperitoneal inoculation of organisms is made. Thus, in one test 3 series of mice were tested with dilutions of a Type I culture in mucin from 10^{-1} to 10^{-5}. One series of mice was given a monotypical Type I antimeningococcal serum, another received normal human serum and the third received no serum at all. All mice receiving no serum or receiving normal serum died, whereas the Type I serum protected in every dilution, that is to say, protected against at least 100,000 minimal lethal doses.

In titrating the protective power of a serum, 2 methods have been adopted. The serum has been diluted out to 1:640 and ½ cc. inoculations of the different serum dilutions have been made one-half to one hour before infection; or the serum dilution and amount inoculated have been kept constant and the time at which the serum inoculation is given has been varied out to 11 hours after the infecting inoculation.

It has been found that the protective value of the serum falls more or less regularly with serum dilution and with the time interval after inoculation. Thus, in a titration of a monotypical Type I antiserum against a virulent Type I strain there was no demonstrable difference between the protective value of serum dilutions 2:5 and 1:10, but a dilution of 1:160 gives significantly less protection and the dilution of 1:640 hardly protects at all. A serum dilution of 2:5 given 30 minutes beforehand is more efficacious in protecting the mice than when given 4 hours and 8 hours after infection, but serum given this late protects against 1,000 M.L.D.

Heterologous antimeningococcal sera give some protection but it is less than that of the homologous serum or polyvalent sera. Thus, Type II serum protects against about 100 minimal lethal doses of Type I organisms when given in a dilution of 2:5 30 minutes before

[4] Webster, L. T., *J. Exp. Med.*, 1933, **57**, 793.

[5] Webster, L. T., *J. Exp. Med.*, 1933, **57**, 819.

TABLE I.

					Type I						Polyvalent X					
					2:5 30 min. before		1:160 30 min. before		2:5 10 hrs. after		2:5 30 min. before		1:160 30 min. before		2:5 10 hrs. after	
	No serum		Normal serum													
10^{-2}	<23	<23	<23	<23	<23	<23	<23	<23	<10	<10	S	S	<23	<23	<10	<23
10^{-3}	<23	23	<23	<23	S	S	26½	<71	<23	<23	S	S	<23	S	<23	25½
10^{-4}	<23	23	<23	24	S	S	<23	<28	11½	24½	S	S	<23	26½	<10	10½
10^{-5}	<23	<23	<23	23	S	S	S	S	23	S	S	S	S	S	<47	S
10^{-6}	<23	25	25½	31	S	S	S	S	S	S	S	S	S	S	S	S
10^{-7}	<47	<71	28	S	S	S	S	S	S	S	S	S	S	S	S	S

1 cc. of each culture dilution given intraperitoneally to each mouse—two in each dilution. ½ cc. of serum given. Dilution of serum and time of inoculation indicated. Time of death in hours indicated. < = mouse died during night in less than the number of hours indicated. S = survival for 5 days.

infection, but fails to protect in a dilution of 1:160 30 minutes beforehand or in a dilution of 2:5 10 hours after infection.

This protection test is being used in the comparison of certain of the polyvalent sera now on the market. All of those tested show good protection (namely against 100,000 m.l.d.) when given in a dilution of 2:5 one-half to one hour before infection. On titrating them, they have been compared with the monotypical Type I antimeningococcal serum prepared in this laboratory. Table I shows such a comparative test. It will be noticed that the normal human serum does not protect. The polyvalent serum gives as good or slightly better protection than does the homologous serum. Both antimeningococcal sera protect when diluted 1:160 (against 1,000 m.l.d.) and when given in a dilution of 2:5 10 hours after infection (against more than 100 m.l.d.).

Thus, the use of Miller's technique has allowed one to titrate the virulence of freshly isolated meningococcus strains and to develop a protection test for titrating antimeningococcal serum. The results in both instances are consistent only when pure breeds of susceptible mice are used. In our hands the use of unselected stock mice has led to results which, on account of their inconsistency, are highly unsatisfactory.

8018 P

Rate of Lymph Flow in Edematous Skin of Cardiac and Renal Disease.

PHILIP D. McMASTER.

From the Rockefeller Institute for Medical Research, New York City.

In normal skin an intradermal injection of deeply colored vital dye renders the lymphatics visible.[1] In a few minutes some of the dye drains away into the deeper channels appearing like colored streamers when seen through the skin. Scores of tests on normal volunteers have shown these colored streamers to be long or short under conditions known to increase or decrease lymph flow respectively. The method, to be described elsewhere, has been used to compare the rate of lymph flow in the edematous skin of cardiac and nephritic patients.

In more than 60 experiments upon 14 individuals with cardiac

[1] Hudack, S. S., and McMaster, P. D., *J. Exp. Med.*, 1933, **57**, 751.

disease and edema of the lower limbs, we have found the lymphatic capillaries widely dilated and dye entered them more readily than in normal skin. The intercommunication between the channels was extremely rich and the injection of the superficial network very complete, showing that they were fully open. Dye was carried farther in the channels to color a wider area and escaped from them more rapidly than in normal skin.

In instances of long standing cardiac edema, isolated colored "islands", skin regions in which the superficial lymphatics contained dye, appeared during the course of the intradermal injection several centimeters away from where the needle had entered and separated from the immediate area of staining by skin of normal hue. These are never seen under normal circumstances. It is plain that some of the injected pigment entered the deeper plexus, passed along this unseen and emerged again at some distance in the superficial plexus. The "islands" appeared below the site of injection as well as elsewhere around it. A valvular incompetence of the deeper lymphatics, due to widening of them, will explain the retrograde flow.

We have never observed a formation of colored streamers in cases of cardiac edema, despite the dilatation of the lymphatic channels—in other words there is none of the evidence of lymph flow seen in the normal limb. Yet the fact that the lymphatics are patent can readily be demonstrated. When a region stained as result of an intradermal injection of dye is massaged, colored streamers promptly appear. If this is done in the case of a patient with outspoken, long standing edema and the skin is stroked from the injection site toward the periphery, a retrograde passage of dye takes place along the superficial lymphatics, sometimes as far as 10-12 cm. The phenomenon is never seen in normal man nor does it occur again in the patient a few days after the edema has been reduced by therapeutic measures. It is indicative of a valvular incompetence of the lymphatics such as will explain the "islands" of dye. In the edematous legs of patients with cardiac disease there is evidently a stagnation of lymph, a true breakdown of lymph transport with failure of the valves to function properly.

In contrast to these findings there exists a greatly increased lymph flow in nephritic edema that is attended by a lowering of the plasma protein concentration. More than 70 experiments upon 16 individuals showed wider lymphatic capillaries than normal but narrower than in advanced cardiac edema. Other local changes are far less pronounced than in cardiac edema and the "islands" of dye

observed in the latter condition, and the other signs of incompetence of the valves have not been encountered.

Practically at once in many cases of nephritic edema, dye streamers become visible extending from the injected region. In every instance they have appeared far sooner, and lengthened much more rapidly than in any normal instance. Within 3 or 4 minutes they have the appearance assumed after 20 minutes to half an hour in the normal human being. Lymph flow is obviously far more rapid than usual. Its rate as judged by streamer formation, varies with the changes in the patient's condition. Streamer formation was least when edema was increasing, but still much more than normal. It was still greater in the stationary periods and greatest by far in periods of diuresis. At these times one or 2 colored streamers developed that extended from the ankle to Poupart's ligament within 15 minutes after an ankle injection of only 0.02 cc. of dye solution in a leg lying horizontally or even extended against gravity to the knee in a leg hanging vertically. Such findings have never been observed in normal individuals.

Lymph flow is excessive in the edematous skin of the nephritic patient. In cardiac edema on the other hand there is a virtual stagnation of the lymph.

8019 P

Relation of Entoptic Stray Light to Flicker and the Perception of Movement.*

S. HOWARD BARTLEY. (Introduced by Geo. H. Bishop.)

From the Oscar Johnson Institute, Washington University, St. Louis.

Under certain conditions, flicker is seen in the visual field surrounding the test-object, presented against a dark background, when this object subtends only a small visual angle. Unlike flicker in the test-object itself, field flicker has received little or no study. It appears when the test-object is very bright, or large, or when the flicker of the test-object is much below its critical fusion frequency. The question arises whether the phenomenon is due either to some sort of neural interaction, or to entoptic stray light. Recently considerable evidence has been adduced to make it entirely

* This work was done under a grant-in-aid for Research in Neurophysiology from the Rockefeller Foundation.

probable that it arises from the latter source. To determine this conclusively, 2 tests were made. Using monocular vision and locating the image of the test-object on the "blind spot" where no neural interaction can take place, field flicker was not abolished. On the other hand, when 2 test-objects equal in all respects are alternately presented on 2 different parts of the visual field, the first disappearing as the second appears, thus keeping the stray light constant, field flicker is abolished, demonstrating its dependence upon actual intermittent illumination rather than upon neural interaction between parts stimulated and those not.

The relation between the critical flicker frequencies of the test-object and the surrounding field was measured under a variety of conditions. With areal increase of the test-object, the c.f.f.'s of both test-object and the field were raised in such a manner as to indicate some interaction between the 2 areas. With increase in intensity, the c.f.f.'s of both were at first raised till a point was reached at which the c.f.f. of the test-object began to fall, so that with further increase in intensity the c.f.f.'s of the 2 areas became identical. Finally the c.f.f. of the test-object became even lower than that of the field. This was true for both foveal and peripheral vision.

When flicker is slow, intermittency of illumination is not the only characteristic of the stimulus but the perception of an apparent alternate expansion and contraction of something within the visual field is added. The test-object first suddenly comes into existence and then expands to its final size, this perceived movement being known to psychologists as *gamma* movement. The present study has shown that it is not confined to the test-object as usually implied, but like flicker occurs also from stimulation by entoptic stray light. In more adequately defining the distribution of light on the retina, a basis for this form of "apparent" movement has been provided in terms of successive response of different parts of the retina which is also essentially the basis for the perception of "real" movement.

8020 P

The Crystalline Ovarian Follicular Hormone.

D. W. MacCORQUODALE, SIDNEY A. THAYER, AND EDWARD A. DOISY.

From the Department of Biological Chemistry, St. Louis University School of Medicine.

Due to the low concentration of estrus-producing material in ovaries, we abandoned their use several years ago in favor of the much cheaper and more concentrated source, namely, the urine of pregnant women and mares. However, with the isolation of so many different estrogenic compounds, it seemed desirable to determine the nature of the active substance in the ovary. Starting last year seriously to work on this problem we soon obtained the hormone in a crystalline condition but owing to the very low concentration in hog ovaries, have not secured enough to complete our work.

Preliminary assays of the crystalline follicular hormone give the following data: (1) ovariectomized mice by the Marrian-Parkes procedure, 200,000 units and by the Butenandt procedure, 70,000 units per mg.; (2) ovariectomized rats by our usual procedure, 16,000 units per mg.; (3) immature rats by the Curtis-Doisy procedure, 5,000 units per mg. In our laboratory these results are from 4 to 8 times the values that we obtain for theelin, but with the immature rat the potency is equal to that of theelol. The assays for dihydro-theelin and for the follicular hormone by the respective methods give similar values.

Although we have not yet accumulated sufficient material for complete analysis, our results indicate the identity of the hormone with dihydro-theelin. The m-bromobenzoate of the hormone was prepared, and after 3 crystallizations had a melting point of 154°-155°. After 4 crystallizations the m-bromobenzoate prepared from a sample of pure dihydro-theelin melted at 155°-156°, and the dihydro-theelin obtained from it by saponification with dilute alcoholic alkali melted at 171°-172° after one crystallization. By saponification of the m-bromobenzoate of the hormone in the same manner the crystalline hormone was recovered and found to melt at 170°-171°. All melting points are uncorrected but were taken with a Bureau of Standards long stem thermometer.

8021 C

Influence of Obstruction of the Bowel upon its Strength (Bursting Strength).*

LOUIS SPERLING AND OWEN H. WANGENSTEEN

From the Department of Surgery, University of Minnesota.

Changes in the bowel wall in intestinal obstruction due to increased intraenteric pressure have been emphasized by many investigators. Perforations of the bowel wall due to gangrene are sometimes terminal complications of simple obstruction.

This study was made to compare the bursting pressure of the bowel wall in simple intestinal obstruction with the normal. Cutting[1] reports the breaking point of normal bowel to be between 1000 and 1500 mm. of mercury. Morton[2] reports a pressure of over 500 mm. of mercury as the breaking point. Burt[3] determined the pressure required to rupture the bowel at various levels by the introduction of air. He found that, experimentally, the rectum supports the greatest pressure, and the sigmoid, *ileum,* esophagus, *jejunum,* transverse colon, caecum, and stomach decrease in strength in the order in which they are mentioned.

Loops of ileum and jejunum 6 to 8 inches long were obtained immediately after death from normal dogs (used for other experiments). One end of the loop was connected to a specially constructed mercury manometer (measuring to 1800 mm. of mercury) and the other to an airline source of pressure. The ends of the loop were tied over rubber nipples to prevent as much as possible blowing out of the connections. The pressure (intraenteric) was then gradually increased until the bursting point (explosion) of the bowel was passed.

Similar loops were obtained from 11 dogs with simple low ileal obstruction of 4 to 7 days duration. The loops were obtained as in the normal cases immediately after death (sacrifice of animal for another experiment), and subjected to distension with air in the same manner as the normal loops. The pressure level at which the serosa split, the site of the tearing (mesenteric or antimesenteric border), and the level of pressure at which the bowel finally burst were noted.

*This work was supported by the Medical Research Fund of the Graduate School, University of Minnesota.

1 Cutting, R. A., *Arch. Surg.,* 1928, **17**, 658.

2 Morton, J. J., *Arch. Surg.,* 1930, **21**, 531.

3 Burt, C. A. V., *Arch. Surg.,* 1931, **22**, 875.

The normal ileum in most cases was able to withstand greater degrees of pressure than the jejunum. The ileum resisted pressures from 300 to over 1,000 mm. of mercury. The bursting pressures for the jejunum fell within the same limits, but, with some exceptions, were lower than those of the ileum in the same dog.

In the obstructed series, however, the reverse was found to be true. The jejunum, *i. e.*, that portion of the bowel farthest away and least affected by the obstruction, was the more resistant. The values for the jejunum approached the normal, whereas those of the obstructed segment of bowel (ileum) were far below normal values. In the obstructed specimens (ileum) the serosa split usually at the antimesenteric border at about 100 to 300 millimeters of mercury, and soon burst, whereas in the normal cases the serosa was able to resist usually above 400 mm. of mercury pressure.

TABLE I.
Bursting Pressures for Normal Bowel.

Dog	Press. mm. Hg at which serosa split		Press. mm. Hg at which bowel burst	
	Ileum	Jejunum	Ileum	Jejunum
1	360a	210a	620	400
2	300a	660a	560	660
3	540	400p	540	400
4	480a	440	480	440
5	660a	600a	Ends blew out	
6	540a	610a	" " "	
7	700a	540a	700	540
8	1060a	820a	1060	820
9	560p	640p	560	640
10	700p	780a	700	780
11	300m	220m	660m	500a
12	1080m	400a	1080	820

aAntimesenteric border.
pParamesenteric border.
mMesenteric border.

TABLE II.
Bursting Pressures for Obstructed Bowel.

Dog	Days Obst.	Press. mm. Hg. at which serosa split		Press. mm. Hg. at which bowel burst	
		Ileum	Jejunum	Ileum	Jejunum
1	6	195a		200—End blew out	
2	6	220a	240	230	540
3	7	200	200	280	225
4	7	90a	190a	180a	260a
5	7	200	200	200	200
6	7	200a	500a	280	760
7	5	210a	420a	250m	620m
8	5	360a	560p	480m	560p
9	5	260a	360a	360a	400a
10	6	400m	500	460m	520a

a, p, m, as in Table I.

Conclusions: 1. The bowel wall in simple intestinal obstruction cannot withstand marked increase of intra enteric pressure as well as the normal bowel wall. 2. This is especially true of that portion of the bowel just above the site of obstruction. 3. The most frequent site of tearing of the serosa and rupture is the antimesenteric border of the bowel.

8022 C

Experiments with Poliomyelitis Virus.*

JOHN A. TOOMEY.

From the Department of Pediatrics, Western Reserve University, and the Division of Contagious Diseases, City Hospital, Cleveland, Ohio.

In my series of experiments on poliomyelitis the average length of time elapsing between the intracerebral injection of the virus in the *M. rhesus* monkey and the onset of paralysis was from 5 to 10 days. The clinical effects of motor cell destruction were not noticed for some time, irrespective of the quantity (from 0.25 cc. to 2.0 cc.) and the concentration (from 1% to 10%) of the virus administered. When the motor areas of 2 monkeys were exposed at operation and from 0.25 cc. to 0.5 cc. of a 2% suspension of virus injected directly into their centers no paralysis developed immediately. When larger amounts (from 2.0 cc. to 2.5 cc. of a 2% suspension) were injected in 2 monkeys in the same area, some partial hemiparesis occurred following the recovery of the animals from the anesthetic. The hemiparesis that occurred was fleeting since the animals recovered within 24 hours. It was not due to the virus, but probably to local mechanical effects, since the same amount of homologous blood serum injected in the exact cortical motor area produced a similar slight immediate hemiparesis from which 2 monkeys recovered just as quickly. The quadriplegia which develops after the injection of the virus occurred only after the usual lag interval, *i. e.*, days after the injection.

The cortical areas are very resistant or perhaps less susceptible than other areas of the central nervous system to the virus. The virus is considered to have a predilection for the motor cells of the lumbar enlargement.

* Expenses defrayed in part by a grant from the Marion R. Spellman Fund, The Cleveland Foundation.

What would follow the injection of the virus into the center of those very cells of the lumbar enlargement that are supposedly highly susceptible or least resistant to the virus? When the cord was exposed at operation and 0.1 cc. of a 1% or 10% suspension of virus injected on one side of the cord in 5 animals, there was no clinical evidence of immediate paresis or paralysis; from 3 to 4 days elapsed before poliomyelitis appeared. However, when the same amount (0.1 cc.) of a chemical that actually destroys cells, such as a solution of acetic acid diluted to pH 6, was injected in the same place, a paralysis, which did not spread, developed in the homolateral leg immediately after (2 monkeys). When doses as large as 0.3 cc. of a 1% suspension of virus were injected in that small area of the cord in one animal and 0.5 cc. of a 10% suspension into another, there was immediate paresis or paralysis following the recovery of the animals from the anesthetic. This paralytic effect must have been caused by other than specific factors since the animals thus injected developed poliomyelitis 2 and 5 days after the injection, respectively. It probably was the result of pressure since the injection of the same dose of homologous serum (0.3 cc. or 0.5 cc.) in the same area in 2 monkeys also caused the same type of immediate paralysis, although there was no later extension of the paralysis in the latter animals.

When the vagus nerve was injected with from 0.2 to 0.3 cc. of a 1% suspension of virus and when paralysis followed, which it did not always do, 3 to 18 days elapsed before its occurrence. When the median nerve of the arm was injected with from 0.3 cc. to 0.5 cc. of a 1% suspension of virus, 2 out of 5 animals injected became paralyzed; one the 5th and the other the 6th day following the injection. The other 3 did not develop paralysis. When the virus was injected between clamps or subserosally into the small intestine from 2 to 14 days elapsed before monoplegia or partial paraplegia developed, the usual onset of paralysis coming on within from 2 to 3 days (31 animals).

P. C. B. filtrate (described previously,[1]) when combined with the virus of poliomyelitis and injected into the motor cell area of the cortex in amounts so small that no effect should have been produced, nevertheless, produced paresis almost immediately. The P. C. B. filtrate, like the virus, was in itself innocuous to the monkey, but when the 2 were combined and injected, paralysis resulted. One tenth of a cc. of a 50% dilution of P. C. B. filtrate injected directly into the lumbar enlargements of 3 monkeys produced neither an

[1] Toomey, John A., PROC. SOC. EXP. BIOL. AND MED., 1934, 32, 423.

immediate nor a late paralytic effect. When, however, 0.1 cc. of a combination made up of equal parts of a 2% suspension of virus and P. C. B. filtrate was introduced into the same area in 3 other animals, an immediate paresis or paralysis occurred in the leg of the homolateral side. There was no quiet period during the interval between the injection of the virus and the production of paralysis. The paralysis of the homolateral leg spread gradually and without a lag to the opposite leg, until it finally involved all the muscles usually affected in quadriplegia.

8023 P

Vitamin B_1 and B_2 Content of Human Urine.

O. M. HELMER. (Introduced by K. K. Chen.)

(With the assistance of Mary Rickards Richardson and Marian Wheeler).

From the Lilly Laboratory for Clinical Research, Indianapolis City Hospital, and the Department of Medicine, Indiana University School of Medicine.

With the hope of throwing some light on the fate of vitamins B_1 and B_2 in the body, preliminary experiments have been made to determine whether human urine contains demonstrable amounts of vitamins B_1 and B_2. As yet there are no satisfactory chemical methods available for these vitamins such as are now in use for determining vitamin C. Therefore biological assays for the vitamin B_1 and B_2 content of the urine were made by the rat feeding technique.

Since it was impossible to fractionate the urine in any way without loss of vitamin, the 24 hour urine samples were concentrated by vacuum distillation and dried *in vacuo* over sulphuric acid. The dried and powdered urine was thoroughly mixed with one part by weight of sucrose and one-half part by weight of Crisco, and fed to rats on a basal diet in quantities equivalent to 1/25 of the daily 24 hour urine output. In the test for vitamin B_1 the basal diet was supplemented with 500 mg. of autoclaved yeast. For the vitamin B_2 experiments the source of the vitamin B_1 was an extract of rice polishings made according to Rosedale.[1] The rats were fed the urine preparations after a depletion period in which the weight had become stationary for 3 weighing periods of 2 days each.

[1] Rosedale, J. L., *Biochem. J.*, 1927, **21**, 1266.

TABLE I.

Experimental Animal	Supplement	No. of Rats	Days Fed	Aver. Total Gain in Wt. gm.	Aver. Weekly Gain in Wt. gm.
+ control	500 mg. yeast	4	28	74.5	18.7
+ "	1 cc. B_1 extract + 500 mg. autoclaved yeast	2	24	34.0	10.5
— "	500 mg. autoclaved yeast	8	43	7.4	1.2
— "	1 cc. B_1 extract	4	49	1.0	0
B_1 normal urine	500 mg. autoclaved yeast + urine	8	27	20.8	6.1
B_2 " "	1 cc. B_1 extract + urine	4	28	28.2	7.1
B_1 pellagra urine	500 mg. autoclaved yeast + urine	1	24	20.0*	5.8
B_1 " "	500 mg. autoclaved yeast + urine	1	24	—2.0	—1.0
B_2 " "	1 cc. B_1 extract	1	20	1.0	0
B_2 " "	1 cc. B_1 extract	1	24	9.0	2.6

*In dying condition in spite of gain.

The urine used for these experiments was collected from 3 normal subjects who ate a weighed amount of a well-balanced diet of 2750 calories per day and which, according to present knowledge, contained adequate amounts of vitamins B_1 and B_2. The urine from one patient with untreated pellagra was also collected.

The results (Table I) indicate that the amount of urine equivalent to 1/25 of the daily output of a normal subject contains demonstrable amounts of vitamins B_1 and B_2. These values are minimal values, for the data on rats which failed to eat all of the urine supplement were not excluded from the table. At the end of the experimental period the rats fed the normal urine were in good condition, whereas the negative controls and the rats fed urine from a patient with pellagra were dead or in a dying condition.

We feel these experiments demonstrate that it is possible to determine the vitamin B_1 and B_2 content of human urine by rat feeding experiments, thereby possibly providing a means of studying the physiology of these vitamins and a method of determining the dietary requirements of these vitamins for man under normal and pathological conditions. Balance studies in normal and diseased conditions are now in progress.

8024 C

Morphologic and Quantitative Reaction of Ant. Pituitaries of Castrated Female Rats to Oestrin Injections.*

J. M. WOLFE.

From the Department of Anatomy, Vanderbilt University School of Medicine, Nashville, Tennessee.

It is well established in the literature that castration in the female rat results in an increase in the basophiles and their development into castration cells. However, there is considerable disagreement concerning changes in the eosinophiles. In recent quantitative studies Ellison and Wolfe[1] reported that castration in the mature female rat resulted in an early increase in the size and the relative percentages of the basophiles in the anterior hypophysis which reached an apex approximately 30 days after castration (Table I). At this time a few signet-ring castration cells appeared. From the 30th day of castration there was an increase in the relative level of the castration cells, while the percentages of the basophiles decreased. There was also a moderate increase in the percentage of the eosinophiles in 30- and 60-day castrates (Table I).

It is equally well established that the injection of oestrin prevents the changes which ordinarily occur in the basophilic elements after castration. The experiments recorded below were carried out in order to obtain statistical data on the action of oestrin on the anterior lobes of female rats castrated for periods of 30 and 60 days.

Seventy-one mature female rats castrated for 30 days and 30 rats castrated for 60 days were used. Twenty-eight of the 30-day castrates received 25 units of oestrin†‡ daily throughout the castration period while 12 of the 60-day castrates received similar injections for the last 30 days of the 60-day castration period. Vaginal smears were made throughout the injection period. Serial sections of the pituitaries were cut and cell counts made; their relative per-

* These studies were aided by a grant from the Division of Medical Sciences of the Rockefeller Foundation.

1 Ellison, E. T., and Wolfe, J. M., *Endocrinology*, 1934, **18**, 555.

† The oestrus-inducing extract, Amniotin, was furnished by E. R. Squibb & Sons through the courtesy of Dr. J. J. Durrett.

‡ A group of 4 rats (30-day castrates) received 200 rat units daily of Progynon B for this period. The pituitaries of this group of rats were greatly increased in weight; the range was from 19 to 24 mg. The pituitary weights of the rats receiving smaller amounts of oestrin (Amniotin) were within the limits for normal female rats.

TABLE I.

Frequency distribution table, giving detailed quantitative data on the levels of the various cell types in normal female rats and in the oestrin-injected and the non-injected groups. Class intervals, means, and standard deviations are given in percentages.

Class Intervals in %	Frequency Distribution in: Normal Controls	30-day Castrates Controls	30-day Castrates Injected	60-day Castrates Controls	60-day Castrates Injected	Means (M) and Standard Deviations (SD) in %: Normal Controls	30-day Castrates Controls	30-day Castrates Injected	60-day Castrates Controls	60-day Castrates Injected
Eosinophiles:										
15.-19.9			1							
20.-24.9	4		3		1					
25.-29.9	14		8		6	M 34.2	M 41.5	M 29.2	M 42.5	M 29.2
30.-34.9	66	1	16		5	SD 4.3	SD 3.9	SD 3.5	SD 2.8	SD 3.1
35.-39.9	49	16		3						
40.-44.9	9	17		12						
45.-49.9	1	9		3						
Basophiles:										
0.- 1.9			7							
2.- 3.9	45		10		1					
4.- 5.9	74		6		7					
6.- 7.9	14		2	1	4					
8.- 9.9		2	3	1		M 4.1	M 13.4	M 3.9	M 13.5	M 5.5
10.-11.9		7		1		SD 1.3	SD 1.9	SD 2.4	SD 2.7	SD 0.9
12.-13.9		18		7						
14.-15.9		12		6						
16.-17.9		4		1						
18.-19.9				1						
Castration Cells:										
None	143	10	28							
- 1.9		28			7					
2- 3.9		3		10	3					
4.- 5.9		1		7	2					
6.- 7.9		1		1			M 0.9		M 4.0	M 2.2
8.- 9.9							SD 1.2		SD 1.2	SD 1.2
10.-11.9										
Chromophobes:										
30.-34.9				2						
35.-39.9		10		6						
40.-44.9		11		7						
45.-49.9	1	16		3		M 61.8	M 45.0	M 66.2	M 40.5	M 63.2
50.-54.9	4	6				SD 4.4	SD 5.0	SD 5.2	SD 4.3	SD 4.3
55.-59.9	41		3		2					
60.-64.9	69		8		5					
65.-69.9	28		13		4					
70.-74.9			1		1					
75.-79.9			3							
Total in Groups	143	43	28	18	12					

centages were calculated and are presented statistically in Table I. For non-castrate control material previous studies on the pituitaries of 143 normal female rats were utilized.

Analysis of table I shows that oestrin injections throughout the 30-day castration period either completely or incompletely prevented the increase in the number and the size of the basophiles. No castration cells were found (Table I). The degree of this prevention of castration changes was proportional to the degree to which the vagina was kept cornified. In many rats the relative percentage of the basophiles was even lower than that found in normal females (Table I). Most of the basophiles were small, regressive and devoid of granules. Oestrin injections also prevented the moderate increase in the percentage of the eosinophiles which ordinarily occurs following castration. Moreover, many of these cells were swollen and exhibited loss of granules. In such cells the negative image of the Golgi apparatus was often enlarged. The relative percentages of the chromophobes were increased (Table I). Many were greatly increased in size; in these large chromophobes as well as in many of the smaller the negative image of the Golgi apparatus was enlarged.

In the 60-day castrate rats receiving oestrin for the last 30 days of this period, the pituitary findings were similar to those in the injected 30-day castrates. Both basophiles and castration cells were found but at a much lower level than in the controls (Table I). The basophiles and, in some instances, the castration cells were small and regressive. The relative percentage of the eosinophiles was much lower than that found in the 60-day castrate controls and the mean level of these cells was slightly lower than that found in the non-castrate controls (Table I). Many of the eosinophiles exhibited loss of granules.

These studies indicate that oestrin injections prevent the differentiation of new basophiles which normally follows castration in the female rat. They fail to indicate the exact action of oestrin on the basophiles known to be present at the beginning of the injections. The moderate increase in the level of the eosinophiles which ordinarily occurs following castration is prevented and many of these cells show loss of granules.

8025 C

Reaction of Anterior Pituitaries of Mature Female Rats to Injections of Large Amounts of Oestrin.*

J. M. WOLFE.

From the Department of Anatomy, Vanderbilt University School of Medicine, Nashville, Tenn.

We have found[1] that injections of extracts of pregnancy urine markedly increased the weight of the ovaries and the pituitaries of female rats. The ovaries contained many large corpora lutea and both partially luteinized and unluteinized follicles. The vaginae were usually mucified at autopsy. The anterior pituitaries exhibited marked loss of granules from practically all basophiles and a decrease in their relative percentage. Many eosinophiles were swollen and showed loss of granules; they were reduced in percentage. The degree of change in the eosinophiles was proportional to the increase in weight of ovaries and pituitaries. Oestrin stimulates the production of a luteinizing hormone by the anterior hypophysis[2, 3] and injection of oestrin into mature female rats results in the formation of large corpora lutea comparable to those of pregnancy.[4, 5] This paper is concerned with the anterior pituitaries of 30 mature female rats which received daily injections of 200 rat units of oestrin† for 12 days and in whose ovaries intense luteinization had been induced. Serial sections of the pituitaries were cut and cell counts made on representative sections. Previous studies on the anterior pituitaries of 143 normal female rats served as controls for these studies. The data on both groups are arranged in a frequency distribution table and analyzed statistically (Table I).

The pituitaries of the injected rats were greatly increased in weight (previously reported by Hohlweg[4]); the mean was 20.1 mg., the range from 12 to 27 mg. The mean pituitary weight of 143 normal cyclic females was 10.5 mg. (Table I). Marked morphologic changes were found in the anterior pituitaries; most outstanding of these was the complete loss of granules from all the baso-

* These studies were aided by a grant from the Division of Medical Sciences of the Rockefeller Foundation.

1 Wolfe, J. M., *et al.*, *Anat. Rec.*, 1934, **60**, 357.

2 Hisaw, F. L., *et al.*, *Anat. Rec.*, 1934, **60** (sup.), 53.

3 Lane, E. C., *Am. J. Phys.*, 1935, **110**, 681.

4 Hohlweg, W., *Klin. Woch.*, 1934, **13**, 93.

5 Wolfe, J. M., Proc. Soc. Exp. Biol. and Med., 1935, **32**, 757.

† Progynon-B was used; a portion of this material was furnished gratuitously by the Schering Corporation, Bloomfield, N. J.

TABLE I.

Summary of quantitative data on control and experiment groups. Class intervals, means (M), and standard deviations (SD) are given in percentage.

Class Interval	Frequency Control	Frequency Experimental	Mean—Standard Deviation Control	Mean—Standard Deviation Experimental
Eosinophiles:				
15.-19.9		3		
20.-24.9	4	17		
25.-29.9	14	10	M 34.2	M 23.6
30.-34.9	66		SD 4.3	SD 3.1
35.-39.9	49		Cells per section—304	Cells per section—308
40.-44.9	9			
45.-49.9	1			
Basophiles:				
0.- 1.9		27	M 4.1	M 1.2
2.- 3.9	55	3	SD 1.3	SD 1.9
4.- 5.9	74		Cells per section—38	Cells per section—14
6.- 7.9	14			
Chromophobes:				
45.-49.9	1			
50.-54.9	4			
55.-59.9	41		M 61.7	M 75.2
60.-64.9	69		SD 4.4	SD 3.4
65.-69.9	28	1	Cells per section—554	Cells per section—983
70.-74.9		12		
75.-79.9		14		
80.-84.9		3		
Mean Pituitary Weight	10.8 mg.	20.1 mg.	Total Cells Counted per Section 896	1305
Mitoses per section	1.6	36.6	—	—

philes. The non-granular basophiles remaining in the gland were considerably enlarged. We have followed the same procedure in making all cell counts.[6] The quantitative data indicate that, in the injected rats, both the relative percentage and the total numbers of the basophiles were reduced below the normal (Table I). Less conspicuous changes were found in the eosinophiles. Many were swollen and exhibited loss of granules. The negative image of the Golgi apparatus of these cells was often enlarged. The relative percentage of the eosinophiles was reduced more or less in proportion to the degree to which the pituitary was increased in weight (Table I). Although the relative percentage of the eosinophiles was reduced, our quantitative data indicate that the total number of these cells counted in the pituitaries of the injected rats was no lower than that found in the glands of normal rats. This condition was due to the large number of mitoses in the chromophobes which increased their absolute number as well as their relative percentage. However, many mitoses were also found in the eosinophiles, which most probably kept the absolute number of these cells near the

[6] Wolfe, J. M., *et al.*, *Amer. J. Anat.*, 1934, **55**, 363.

normal in spite of the fact that many were losing their granular material and gave rise to chromophobes.

The chromophobes were greatly increased in relative percentage and in the numbers counted (Table I). Mitoses were abundant (approximately 4 to 5 times as many as occurred in the eosinophiles). Many of these cells were greatly swollen. In some the cytoplasm stained light blue or was almost colorless; in such cells the cytoplasm was often fragmentary. In others of the enlarged chromophobes the cytoplasm was more dense and stained a deeper blue. Other chromophobes were smaller and had a dense blue cytoplasm. In both the large and small chromophobes the negative image of the Golgi apparatus was often increased in size. Often small yellowish masses were found in the region of the Golgi apparatus.

In the glands which were markedly increased in size, the vascularity was also increased; the capillaries in many regions were extremely dilated and greatly emphasized the cord-like arrangement of the anterior lobe cells. The increased size of the pituitary was due to several factors, among which was the increased vascularity, the swollen condition of many of the cells, and the increased number of the cells. Vaginal smears were made daily throughout the experimental period. The smears were usually completely cornified for the first 4 or 5 days, but with 2 exceptions the cornified epithelium failed to persist throughout the entire injection period. At autopsy the vaginal epithelium of 16 rats was mucified; that of the others was stratified and in 2 instances also cornified. These findings suggest that the direct effect of the oestrin on the vagina was partially nullified by the hormone of the corpus luteum produced in the animal's own body. This is in agreement with the recent work of Allen and Meyer.[7]

The weight and morphologic reactions of the anterior pituitaries of mature female rats to pregnancy urine extracts and to oestrin were strikingly similar. In both, the pituitaries were greatly increased in weight. Considering the groups as a whole, morphologic changes were more marked and constant in the rats receiving oestrin. Changes in the eosinophiles were identical in both instances: swelling of the cells, loss of granules, and reduction in relative percentage. This strongly suggests that the oestrin produced in the body of the rats which received pregnancy urine extract was responsible for the changes in the eosinophiles. In previous papers con-

[7] Allen, W. M., and Meyer, R. K., Abstract 4202, Wistar Institute Bibliographic Service.

cerned with the cyclic morphologic changes in the anterior lobe, we have reported that the eosinophiles exhibited loss of granules only when active corpora lutea were present in the ovaries. The findings reported here are similar and probably indicate that the changes found in the eosinophiles during the lutein phase of the normal oestral cycle were due to the action of oestrin. Although oestrin stimulates the luteinizing hormone and at the same time induces granular loss from the eosinophiles, there is at present insufficient evidence to make any definite statement concerning the significance of this finding. Severinghaus[8] has also reported changes in the eosinophiles of the mature female receiving extracts of pregnancy urine, and Nelson[9] found degranulated eosinophiles in rats following injections of oestrin.

The basophiles of rats receiving oestrin and of those receiving extracts of pregnancy urine were similar in appearance. This was especially striking in the anterior lobes of immature rats receiving extracts of pregnancy urine or oestrin. In both instances many large clear blue non-granular basophiles were found. Whether the fundamental factor acting on the basophiles in the rats receiving extracts of pregnancy urine was oestrin is at present not known. However, it is interesting to note that extracts of pregnancy urine do not have any action on the anterior lobes of castrated female rats, while oestrin is capable of direct action on the anterior pituitary. Furthermore, since extracts of pregnancy urine are capable of inducing the formation of oestrin in the ovary of the hypophysectomized female rat, the fact is suggested that oestrin is the effective agent in inducing the above basophilic changes in the anterior lobes of intact female rats receiving extracts of pregnancy urine.

---

8 Severinghaus, A. E., *Anat. Rec.*, 1934, **60**, 43.

9 Nelson, W. O., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 452.

8026 P

Cell Types Found in the Harding and Passey Mouse Melanoma Grown in vitro.

C. G. GRAND. (Introduced by Robert Chambers.)

From the Department of Biology, Washington Square College, New York University.

The growing of melanoma tissue *in vitro* has presented a good method for identifying the various cells found in this tumor. In sections of the tumor the study of the cells is more difficult because of the heavy pigmentation. In tissue cultures cells migrate from the margin of the explant, retain their specific morphology and are easily identified.

Previously, attempts had been made to grow this tumor *in vitro* but without success. At the beginning of our experiments we found that the fragments of explants rendered the medium alkaline and as long as this alkalinity was maintained no growth occurred. We found that we could best counteract the alkalinity by implanting pieces of normal tissues in the same medium. The growth of the normal tissues produces an acidity sufficient to counteract the alkalinity. Under these conditions good healthy growths were obtained. It was also found that fragments of the tumor will grow by themselves, provided that the margins of the fragments contain relatively non-pigmented macrophages because these cells begin to migrate and their continuous growth causes the medium to become less alkaline.

Three types of cells appear in the outgrowths—macrophages, fibroblasts and melanoblasts. The macrophages can be differentiated into actively moving, sparsely laden cells, and swollen, sluggish cells densely filled with melanin granules. The fibroblasts have oval nuclei and are frequently spindle-shaped. The melanoblasts have spherical nuclei and are highly dendritic and are of 2 kinds morphologically: small cells with slender, uniform dendrites, and large cells with stouter dendrites possessing knobbed swellings along their lengths.

The melanin granules in the various types of cells are distributed as follows: In the macrophages they are usually packed in irregular clumps throughout the entire protoplasm; in the fibroblasts they are massed about the nucleus and are sparse or absent in the pseudopodia; in the melanoblasts they occur in the dendritic processes and in the periphery of the main cell body. The granules are fine and

sparse in the dendrites of the small melanoblasts while in the large melanoblasts they are more numerous and tend to be collected into clumps, especially in the knobbed swellings.

The melanoblasts gave a positive reaction to the dioxyphenylalanine (Dopa reaction). Mitoses of the small type melanoblast were observed. No epithelial outgrowth has ever been found in the cultures we have studied.

8027 P

Permeability of the Nuclear Membrane to Vital Stains.

LUDWIK MONNÉ. (Introduced by Robert Chambers.)

From Washington Square College, New York University.

The staining of the nucleus has been considered in general as a sign of decreased vitality of the cell leading to its death. In spite of that we find in the literature a few reliable data indicating that vital staining of the nucleus may occur. This has been accomplished by immersing living cells in solutions of various dyes, both of plants and of animals. It has also been accomplished by micro-injecting various dyes, especially the sulphonated pH indicators of Clark and Lubs into amoebae and tissue culture cells. The stain blown against the nuclear membrane is taken up by the nucleus, but after a short time the color disappears. The possibility at least of a transitory vital staining of the nucleus seems to be fairly well established.

It was of interest to note whether the nucleus is stainable by any dye or only by certain groups of dyestuffs, that is to say, the question was: whether the nuclear membrane is selective or freely permeable. In order to test this it was necessary to micro-inject directly into a cell, since it is well known that many dyestuffs do not penetrate living cells from the environment.

Amoeba dubia and *A. proteus* were selected for the experiments. Aqueous solutions of the stain were blown by means of a micropipette into the cytoplasm directly against the nucleus without injuring its wall. The staining of the nucleus was considered as vital only when the amoeba recovered completely. When the amount injected was very small it was possible to get the staining of the nucleus only, but when the amount was larger the surrounding cytoplasm was also stained. About 40 different dyes were

used and in all cases in which a vital staining of the nucleus occurred the color was transitory, fading away sooner or later.

Vital staining of the nucleus was obtained with dyes falling into the following groups*: 3 nitro dyes, 7 azo-dyes, 5 thiazins, 4 oxazins, 1 amido-azin, 1 safranin, 1 diamino-triphenylmethane, 4 triamino-triphenyl-methanes, 2 amino-hydroxy-triphenylmethanes, 1 pyronin, 1 rhodamin, 3 fluoran derivatives, 4 sulphonphthaleins, 1 acridin. No dye was found that would not penetrate the nuclear membrane by injecting the amoeba close to the nucleus. The following dyes, not included in the above list, in the most diluted solution, also penetrated the nuclear membrane, but recovery occurred only when a small part of the nucleus was stained: Water blue, Azo blue, Methyl blue, Alizarin red, and Ruthenium red. Such dyes as Sodium carminate, Trypan red, Nigrosin, Vital red HR, Rosindulin GG and Congo red always kill when they enter the nucleus, even in the most diluted solution.

The listed dyes are basic or acid, used in neutral, basic or acid solutions, lipoid soluble or lipoid insoluble, crystalloidal or colloidal, organic or inorganic compounds. It is of interest to note that these dyes gave vital staining of the nucleus only when injected. By immersing amoebae into the dye solutions no staining of the nucleus occurred even when the dye was able to penetrate the cell. The stain fades within a few seconds (*e. g.,* some acid dyes) to at least 15 minutes (*e. g.,* Toluidine Blue). The length of time depended largely upon the concentration, the amount injected and the acidity or basicity of dyes. The coloration with acid dyes progresses and disappears quickly. In general, basic dyes penetrate the nucleus in an advancing wave and the color disappears slowly. The decoloration of a nucleus stained with basic dyes inside the cytoplasm proceeds faster than that of an isolated nucleus stained outside the cell in the culture medium. Dyestuffs having a complicated chemical structure are more toxic than those having a simple one. We may roughly say that dye molecules having many benzene rings are more toxic than those having few. But besides that we must take other factors of toxicity into account.

An irreversibly injured nucleus is always eliminated or pinched off. When, during the pinching off process, the irreversibly injured nucleus was prevented from passing out by holding it back with a microneedle, first, only the injured cytoplasmic part was thrown out, but afterwards the nucleus was also pinched off.

* Classification of the dyes following Conn, Biological Stains 1929.

We conclude that the nuclear membrane is freely permeable to a great variety of substances both crystalloidal and, at least, fine colloidal. How far this may be generalized further researches will show.

8028 P

Disposal of Dyes by Proximal Tubule Cells of Chick Mesonephros in Tissue Culture.

ROBERT CHAMBERS.

From Washington Square College, New York University.

Dyestuffs have been used extensively for studying renal activity. However, from evidence in the literature there seems to be no rule governing intake of the various kinds of dyes by the tubule cells. For example, some lipoid-soluble dyes, to which cells are known to be permeable, are not passed into the tubular urine while, of the lipoid-insoluble dyes, some are known to be passed into the urine and others are not, with no apparent reference to their chemical constitution.

By means of the tissue culture method we have been engaged in testing the behavior of the proximal tubules to a series of dyes. Aqueous solutions of the dyes in various concentrations are mixed with the usual tissue culture medium in which are planted fragments of the functioning mesonephros of a 9-11-day chick. This method affords a means of studying the problem in a more direct manner than hitherto possible. Moreover, it enables one to restrict the problem to the proximal tubules which remain alive and functional for days in the explant. Isolated segments of the tubules regenerate their cut ends and become converted into closed sacs into which the progressive accumulation of color can be observed microscopically.

In this report the results of experiments are given on the use of the following dyes. They are the lipoid-soluble basic dyes, Neutral red, No. 825; and Nile blue sulfate, No. 913; which are in general use as vital stains, and the lipoid-insoluble acid dyes, Xylene cyanol FF, No. 715*; Amaranth, No. 184; Acid fuchsin, No. 692; and Orange G No. 27. These acid dyes resemble the sulphonephthaleins in forming highly dissociated, sulfonated compounds in

* The numbers appended to the names of the dyes are those given in Rowe's Colour Index, 1st edition (Society of Dyes and Colourists, Bradford, Yorkshire).

aqueous solutions but differ from them in not being passed by the tubular epithelium into the tubular urine.

It was found that the acid dyes, although they do not vitally stain the cells in general, nevertheless will color the proximal tubule epithelium in such a way that, in the early stages, the color closely resembles the usual vital stains. However, in their staining ability these dyes differ radically from Neutral Red and Nile Blue Sulfate in at least 2 ways.

First, the fine granule-like, colored bodies in the cells coalesce in time to form deeply stained vacuoles which vary in size and may become so large as to occupy the greater part of the interior of the cell. The size of these vacuoles varies with the different dyes used, for example, with cyanol, the vacuoles tend to remain small while those produced with orange G and with Acid fuchsin become so large that the protoplasm of the cell with its nucleus may become converted into a thin peripheral layer similar to the protoplast which encloses the large sap vacuoles in typical plant cells.

Secondly, cold (3-6°C.), which lowers the metabolic activity of the epithelium and prevents the passage of the sulfonephthaleins into the tubules,[1] suppresses the coloration of the cells by the acid dyes. On the other hand this low temperature has no effect on vital staining with the basic dyes.

The results of these experiments are of interest because they show that the cells of the proximal tubules may take up dyes in one of 2 ways. One way appears to be a passive infiltration of a lipoid soluble dye which takes place regardless of temperature changes within viable limits and the other, which depends upon the metabolic activity of the cells. The experiments also show a definite relation between the segregation of fluids into intracellular vacuoles and that type of renal secretion which consists in the transference of materials through the cells into the lumina of the tubules.

It has been shown that a group of sulfonated dyes which are actively taken up by renal cells can be arranged in a series according to the manner in which the cells finally dispose of the dye. At one end of the series are those which pass right through the cell into the lumina of the tubules without any sign of being segregated, even temporarily, within the cell. At the other end of the series are those which are collected in segregation vacuoles in the cells and very little, if any, is passed on into the lumina of the tubule.

[1] Chambers, R., and Kempton, R. T., *J. Cell. and Comp. Physiol.*, 1933, **3**, 131.

8029 C

Influence of Bile Salts on the Nervous System Following Intraspinal Usage.*

S. S. LICHTMAN AND E. L. STERN. (Introduced by George Baehr.)

From the Laboratories and the Medical Services, The Mount Sinai Hospital, New York.

Few observations have been reported concerning the influence of bile and bile salts on nerve tissue.[1] Nerve conduction is not affected but the neuromuscular junction and reflex centers of the cord are influenced by their toxic action.[2] In human subjects it has been claimed that jaundice exercises an analgesic effect on pain.[3] The pruritus of icteric individuals has been linked with a disturbance in the sympathetic nervous system.[4] In both instances, bile salts have been thought to be responsible.

The intraspinal introduction of bile salts was investigated for the purpose of determining whether they exercise an analgesic effect which might be employed for the relief of intractable pain. Clinical experience with the intraspinal use of alcohol for this purpose has been reported by one of us.[5]

Spinal puncture was performed on young lean cats in the lumbar region. Traumatization of the cord at the site of injection frequently occurred judging from the gross pathological findings. In 5 of 105 cats a spontaneously free gush of cerebro-spinal fluid was obtained immediately upon the introduction of the needle. In 28, free fluid could be aspirated. Cisternal puncture was performed in 5 animals.

Motor changes and sensory responses to pin prick were noted at frequent intervals over varying periods of time. Animals were sacrificed under ether anesthesia.

The sodium salt of desoxycholic acid (Riedel-de Haen) was employed because of its purity and great toxicity for pneumococci and erythrocytes.[6]

* Aided by a grant from the Sandoz Chemical Co.

1 Biedl, A., and Kraus, R., *Cent. f. Inn. Med.*, 1898, **19**, 1185; Loewit, *Prag. Z. f. Heilk*, 1881, **2**; Cit. by Meltzer, S. J., and Salant, W., *J. Exp. Med.*, 1906, **8**, 127; Pritzker, B., *Dtsch. Z. f. Chir.*, 1934, **243**, 85.

2 Ries, F. A., and Still, E. U., *Am. J. Physiol.*, 1930, **91**, 609.

3 Hench, P. S., *Ann. Int. Med.*, 1934, **7**, 1278.

4 Lichtman, S. S., *J. A. M. A.*, 1931, **97**, 1463.

5 Stern, E. L., *Am. J. Surg.*, 1934, **25**, 217.

6 Lichtman, S. S., *J. Biol. Chem.*, 1934, **107**, 717.

In a control group of cats, 95% ethyl alcohol was introduced into the spinal canal. In a second series, phosphate buffered desoxycholate solutions were used, and in a third, alcoholic solutions of desoxycholate.

95% *Ethyl Alcohol.* 14 cats were studied in this series. 0.5 cc. could be introduced into the lumbar region with no ill effects. In larger amounts both sensory and motor changes were produced.[7] Via cisternal puncture the injection of 0.1 cc. resulted in slight ataxia lasting 15 minutes, but no gross changes occurred in 13 days. The injection of 0.5 cc. by this route caused prompt respiratory paralysis and death.

Phosphate Buffered Desoxycholate Solutions. Eleven cats were studied in this series. Concentrations between 0.010 and 5.0% in tenth molar phosphate buffer solution pH 7.0, were tested in doses from 0.1 to 3.0 cc. One cc. of 5.0% caused salivation, rapid breathing, incontinence, and death in 30 minutes from respiratory paralysis; one cc. of 0.5% solution caused more marked motor than sensory changes and death in 7 days. 0.5 cc. of 0.5% and of 1.0% solution caused no motor or sensory changes over a period of 14 days.

Desoxycholate in 95% Ethyl Alcohol. Twenty-six cats were studied in this series. Concentrations between 0.010 and 5.0% were injected. 0.1 cc. of 0.010% caused no changes for as long as 15 days. Minimal gross changes were observed on the surface of the cord. Injection of 0.5 cc. of 0.1% caused loss of sensation and motor paralysis of one hind limb and bladder paralysis in one cat. After 10 days a marked hemorrhagic reaction in the lumbar and caudal regions was found. 0.5 cc. of 0.2% desoxycholate caused loss of sensation of all limbs and motor paralysis of the hind limbs, also bladder paralysis. Seven days after injection a hemorrhagic reaction over the entire cord was found.

In cats with an unusually free flow of fluid immediately upon introduction of the needle into the lumbar region, the injection of 0.3 cc. of 2.0% desoxycholate and 5.0% desoxycholic acid did not cause the slightest motor or sensory change. After 30 days no gross change could be detected in the cord. Introduced via cisternal puncture, as little as 0.1 cc. of 0.010% desoxycholate in alcohol caused weakness and ataxia of the hind limbs. After 24 hours a plastic arachnoiditis was found.

Influence of Spinal Fluid and Spinal Cord Tissue on the Hemolytic Action of Bile Salts. Employing the hemolytic action of bile

[7] Stern, E. L., in press.

salts as a possible criterion of toxicity, the influence of the protein content of spinal fluid and of cord tissue upon the action of bile salts was tested. Pooled human spinal fluid, spinal cord of a cat, and a standardized sheep erythrocyte suspension and hemolytic system[7] were used. The presence of spinal fluid in the system necessitated a 40% increase (from 0.010 to 0.014%) of desoxycholate to produce complete hemolysis in a specified time. The further addition of equal segments of spinal cord into the system necessitated a 100% increase, *i. e.*, from 0.010% to 0.020% desoxycholate.

Pathological Findings. Material selected from each of the experimental groups was studied with various staining methods. Where definite fat changes were demonstrable, unequivocal evidence of trauma due to puncture was usually present. In one instance, thrombosis of the anterior spinal artery occurred with extensive myelomalacia in the course of its supply. With alcoholic solutions of desoxycholate in higher concentration, hemorrhagic injection of the meninges up to the cervical region was noted. Localized myelomalacia, softening, and swelling at the level of injection was interpreted as traumatic.

The intraspinal injection of 0.5 cc. of ethyl alcohol alone, in the cat, causes very slight functional and pathological changes in the nervous system. In larger doses it produces sensory and motor changes. Amounts less than 0.5 cc. of buffered desoxycholate in concentrations as high as 1.0% are ineffective in injuring nerve tissue. Combined with as little as 0.1 cc. of alcohol, desoxycholate solutions of 0.025% or more, cause definite sensory, motor, gross and microscopic changes if the lumbar cord is injured at the time of injection. Five percent desoxycholic acid in alcoholic solution produced no injury when there was an immediate free flow of spinal fluid without trauma. Apparently spinal fluid protein and intact nerve tissue tend to diminish the hemolytic activity of the bile salt. The combination of alcohol and bile salt in minute doses is highly toxic to injured nerve tissue. The alcohol apparently permits greater diffusion of the bile salt through injured nerve tissue even in the presence of spinal fluid protein.

The results offer little encouragement for the intraspinal use of bile salt combined with alcohol, or of bile salt alone, for the possible relief of intractable pain. The medullary centers are sensitive to the action of bile salts when introduced by cisternal puncture.

Summary. Sodium desoxycholate can be introduced in minute doses in aqueous solution intraspinally in the lumbar region in cats without untoward effects or injury to the cord. Larger doses pro-

duce motor and sensory disturbances, and even death from respiratory paralysis. Traumatized spinal cord tissue is highly susceptible to the toxic action of even minute doses of bile salt in alcoholic solution although apparently resistant to the same doses in aqueous solution. Spinal fluid protein and cord tissue reduce the hemolytic action of bile salt.

8030 P

Digestibility of Gastric Mucin in vivo.

RICHMOND K. ANDERSON AND SAMUEL J. FOGELSON. (Introduced by C. J. Farmer.)

From the Departments of Experimental Surgery and Biochemistry, Northwestern University Medical School.

We have previously reported studies indicating that gastric mucin (hog) is relatively resistant to enzymatic hydrolysis *in vitro*.[1] However, indirect evidence suggests more complete digestion in the gastrointestinal tract.[1] To further investigate the digestion of mucin we have fed purified gastric mucin as the source of nitrogen, to a series of albino rats, and from nitrogen analyses of the urine and feces determined its degree of digestibility *in vivo*.

Our experimental procedures differed little from those commonly employed in the determination of utilization, digestibility and biological value of proteins. Young growing albino rats weighing 40-60 gm. were placed in cages so designed that urine and feces could be collected separately. Nitrogen intake was calculated from the weight of food consumed. The small amount spilled was corrected for by determining its nitrogen content and subtracting from the calculated food nitrogen. All nitrogen analyses were by the Kjeldahl method.

The purified mucin[2] used in the preparation of the mucin diet gave the following analyses: Nitrogen 7.50%. Reduction after acid hydrolysis (Shaffer-Hartmann) 35.4% (as glucose) and ash 2.51%. The diet was designed to be complete for the rat exclusive of its protein content. This necessitated the addition of a small

[1] Anderson, R. K., and Farmer, C. J., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 21.

[2] Anderson, R. K., Fogelson, S. J., and Farmer, C. J., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 518.

TABLE I.

Utilization and Digestibility of Gastric Mucin.

Mucin-Free Diet					Mucin-Containing Diet								
Initial Wt. gm.	Final Wt. gm.	Food Intake gm.	Fecal N. mg.	Urinary N. mg.	Initial Wt. gm.	Final Wt. gm.	Food Intake gm.	Nitrogen Intake mg.	Fecal N. mg.	Urinary N. mg.	Nitrogen Balance mg.	% Utilization	% Digestibility
							Group I.						
62	51	25	51	85	54	57	32	492	178	282	+32	63.8	74.1
49	40	23	48	64	42	43	25½	393	143	272	—22	63.5	75.7
49	42	18	39	78	44	45	26	398	151	256	— 9	62.0	71.8
54	46	20	44	76	46	47	26½	401	149	230	+22	62.8	73.8
47	42	22	41	68	44	44	25½	390	154	226	+10	60.5	71.0
53	44	23	42	69	46	48	28	429	161	254	+14	62.4	72.2
							Group II.						
42	38	16	33	62	38	36	21	320	138	221	—39	56.8	67.2
61	57	16	38	78	57	59	27½	422	159	276	—25	62.3	71.3
45	43	16	38	55	41	40	22½	343	156	211	—24	54.5	65.5
45	42	14	40	62	41	39	24½	375	162	212	+ 1	56.8	67.4

amount of yeast concentrate (Yeast Vitamin-Harris), the amount added being sufficiently small to be disregarded for practical purposes.[3] A preliminary period on a diet similar except for the absence of mucin was employed in order to determine the fecal nitrogen on a nitrogen-free diet, or the so-called metabolic nitrogen. Four-day periods were allowed between changes in diets to insure the attainment of equilibrium. Collection periods were each of one week's duration. The compositions of the diets were as follows:

Mucin Diet		*Mucin-Free Diet*	
Mucin	20%	Dextrinized starch	80%
Dextrinized starch	60%	Fat (hydrogenated cottonseed oil)	15%
Fat (hydrogenated cottonseed oil)	15%	Salt mixture (Osborne-Mendel)	4%
Salt Mixture (Osborne-Mendel)	4%	Sodium Chloride	1%
Sodium Chloride	1%		

To each diet was added yeast concentrate (Yeast Vitamin-Harris) to a concentration of 0.44% and each rat received in addition one drop of haliver oil plus viosterol (Abbott) every second day. The diets were found to contain 1.561% and 0.066% nitrogen respectively (after yeast concentrate addition).

Utilization was calculated as $\frac{\text{N intake}-\text{fecal N,}}{\text{N intake}}$ and digestibility as $\frac{\text{N intake}-(\text{fecal N}-\text{metabolic N}),}{\text{N intake}}$

The table gives data representative of that obtained in a total of 5 collection periods on 2 different groups of rats. While the digestibility was found to be somewhat lower than that reported for most animal proteins, it is considerably greater than we obtained *in vitro* and indicates that in the case of the rat at least, mucin is to a large extent digestible. It will be observed, however, that the rats failed to gain weight, nor was there significant nitrogen retention. These facts indicate low biological value. Other experiments have further confirmed the inadequacy of purified commercial gastric mucin for growth. The source of this deficiency is being investigated.

[3] Mitchell, H. H., *Phys. Rev.*, 1924, **4**, 466.

8031 P

Exophthalmos in Rabbits Produced by Oxyquinoline Sulphate.*

H. R. MILLER AND HARRY TAUB. (Introduced by G. G. Ornstein.)

From the College of Pharmacy, Columbia University, and Montefiore Hospital.

The intravenous injection into rabbits of oxyquinoline sulphate solution (5%) in amounts of 0.8 to 1.5 cc. per kilo leads to bilateral exophthalmos within 1 to 3 minutes, and the degree of exophthalmos depends, as a rule, upon the amount of solution injected. This rapidly induced exophthalmos appears easily and repeatedly in the Dutch and Belgian species whereas many members of the albino species do not respond. The suitable rabbits, however, do not fail to show exophthalmos over and over again upon adequate doses of oxyquinoline.

Within 50 to 80 seconds after the introduction of oxyquinoline the ear veins engorge and are very cyanotic, marked hyperpnea appears, the heart rate is rapid, salivation is marked, and from 1 to 3 minutes later, 2 or more limbs become flaccid. From larger doses, 1.5 to 2 cc. per kilo, the animal may die promptly, or—and this is the rule,—it remains almost motionless, all limbs extended and flaccid, the head turned to one side; occasionally convulsions are seen (2 cases) and occasionally opisthotonos (2 cases). This report covers 15 rabbits tested. There was 1 lethal outcome.

The eye manifestations are as follows and in this sequence, as a rule: cyanosis of the fundi, steady increase in the area of corneal surface visible, progressive showing of the third lid, nictitating membrane, dilatation of the pupil. The exophthalmos lasts from 2 to 10 minutes and the bulging of both eye balls is equal, although occasionally more pronounced on one side; the eyes gradually return to normal, the eye balls receding slowly and the dilatation of the pupils persists for another 1 or 2 minutes.

This type of acute bilateral exophthalmos was also produced in rabbits who had the cervical sympathetic trunk severed on one side. Except for the failure of the pupil to dilate on the side where the sympathetic was cut exophthalmos appeared to be similar to the phenomenon observed in normal rabbits and identical on each side.

We are engaged in a study of the mechanism underlying this acute type of bilateral exophthalmos.

* We are indebted to Dr. Marine and Dr. Rosen for help and advice.

8032

Additional Note on Decomposition of the Group A Substances.

K. LANDSTEINER AND M. W. CHASE.

From the Laboratories of the Rockefeller Institute for Medical Research, New York.

The destruction of the group A substance in horse saliva by the Myxobacterium of Morgan and Thaysen[1] was recently reported[2] as evidence for its carbohydrate nature. Through the courtesy of Drs. Wadsworth and Sickles, we have made similar tests with another of the microorganisms having the property of decomposing bacterial polysaccharides, an organism isolated from soil by Sickles and Shaw and designated *Saccharobacterium ovale.*[3] The results support the previous findings.

The test substances were added to the synthetic fluid medium "S" described by Sickles and Shaw[3] and the organism was cultivated at 32°C. for 2 to 7 days. In the case of pepsin (Fairchild, 1:15,000), a solution was boiled and coagulated protein removed before addition to the basic medium.

The organism was found to destroy the group A substances of horse saliva, human saliva and also that present in commercial pepsin.[4] The A substance in the pepsin was not utilized by the Myxobacterium of Morgan and Thaysen, and in general the microorganism of Sickles and Shaw was the more active. In cultures 2 days old there remained less than 1% of the original serological activity of an 0.08% pepsin solution.

The activity of the solutions was determined in the manner previously described.[2]

---

1 Morgan, W. T. J., and Thaysen, A. C., *Nature*, 1933, **132**, 604.

2 Landsteiner, K., and Chase, M. W., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 713.

3 Sickles, G. M., and Shaw, M., *J. Bact.*, 1934, **28**, 415.

4 Brahn, B., Schiff, F., and Weinmann, F., *Klin. Wschr.*, 1932, **11**, 1592.

8033 C

Influence of Chicken Liver Feeding on Depancreatized Dogs.

F. S. CUTHBERT AND A. C. IVY.

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago.

The observations of Blotner and Murphy[1, 2] seemed to indicate that certain aqueous liver extracts, as well as liver itself, contain a substance which, when given by mouth, reduces the insulin requirement of diabetic patients. More recently, de Pencier, Soskin and Best[3] stated that the findings of Blotner and Murphy were not substantiated by observations on depancreatized dogs. In all of the work done by both of the above groups, calves' liver was used. The fact that chickens and ducks do not develop a typical Von Mering-Minkowski diabetes [4-8] suggested that chicken liver be tried instead of calves' liver. At the same time it seemed to be desirable to repeat the work of de Pencier, Soskin and Best.

Two dogs were totally depancreatized. Two accurately weighed meals were fed each day, the hour being constant at all times. Urine was collected at exactly the same time every day, and quantitative sugar determinations were made by the Benedict method. Minced calf-pancreas was added to the diet. Insulin was given hypodermically before each meal, the dog being standardized to a dosage of insulin which allowed the excretion of a small amount of sugar.

The liver (200 gm.) was added to the control meal, the theory being that if the liver were sufficiently effective to warrant positive results, it would be able to take care of itself as regards its potential carbohydrate content. The control meal consisted of 200 gm. lean meat, 200 gm. calves' pancreas, and 250 cc. of whole milk, and yielded 756 calories. The addition of the liver to this diet gave it a calorific value of 1050. The lean meat used was the same through-

1 Blotner, H., and Murphy, W. P., *J.A.M.A.*, 1929, **92**, 1332.

2 Blotner, H., and Murphy, W. P., *J.A.M.A.*, 1930, **94**, 1811.

3 de Pencier, M. T., and Soskin, S., and Best, C. H., *Am. J. Physiol.*, 1930, **94**, 548.

4 Weintraud, W., *Arch. f. Exper. Path. u. Pharm.*, 1894, **34**, 303.

5 Kausch, W., *Arch. f. Exper. Path. u. Pharm.*, 1896, **34**, 274.

6 Koppanyi, T., Ivy, A. C., Tatum, A. L., and Jung, F. T., *Am. J. Physiol.*, 1926, **78**, 666.

7 Seitz and Ivy, PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 463.

8 Seitz, *Am. J. Physiol.*, 1930, **93**, 686.

out, a large stock being on hand. The composition of the milk and pancreas probably varied slightly daily.

The dogs remained in excellent general condition and consumed all the food given.

The results may be briefly summarized as follows: During a period of 6 days on the control diet the average daily excretion of sugar was 3.1 gm. (body wt. 23 lb., 22 u. of insulin per day). During the subsequent 10 days with liver added to the diet, the average daily excretion of sugar was 7.4 gm. (body weight 26 lb., 22 u. of insulin daily). During the following 10-day period red beef muscle in an amount of approximate caloric value to the liver was substituted for the chicken liver. The average daily sugar excretion was 7.3 gm. (body weight 25 lb., 22 u. of insulin daily). The repetition of this experiment using longer test periods yielded analogous results. The same was true for dog 2, in which one experiment was performed.

On averaging the results of the 3 experiments, the extra-sugar excreted, when 200 gm. of chicken liver were fed, a figure of 10.37 gm. was obtained. However, the calculated glucose equivalent of 200 gm. of chicken liver is 31.6 gm. of glucose. Since a gain in weight occurred on the addition of liver, it was evident that retention resulted. Recalling that Soskin[9] found a retention of as much as 50% of 50 gm. of the glucose fed to depancreatized dogs receiving no insulin, we decided to give our dogs, while on the control diet and "controlled" with insulin, 31.6 gm. of glucose, divided into 2 portions and given with the meals, 9-day test periods being used. When the results of the 2 experiments were averaged, it was found that the administration of 31.6 gm. of glucose yielded only 10.38 gm. of extra urinary glucose, *i. e.*, two-thirds of the extra-glucose fed was retained or unaccounted for by examination of the urine.

These results show that a depancreatized dog "controlled" with insulin does not quantitatively excrete glucose added to the diet or the calculated glucose equivalent of food added to the basal diet. This is not surprising in view of Allan's[10] work showing that the glucose equivalent of a unit of insulin increases up to a certain point on increasing the carbohydrate intake. This, we believe, explains the apparent "insulin-sparing" action of liver. The observation that chicken liver has no "insulin-sparing" action in depancreatized dogs confirms the findings of Pencier, Soskin and Best[3] for calves' liver.

[9] Soskin, *J. Nutrition*, 1930, **3**, 99.

[10] Allan, *Am. J. Physiol.*, 1924, **67**, 275.

8034 P

Parathyroid Enlargement in Rats Following Experimental Reduction of Kidney Substance.

W. A. JARRETT, H. L. PETERS, AND A. M. PAPPENHEIMER.

From the Department of Pathology, College of Physicians and Surgeons, Columbia University.

There is evidence from human pathology that renal disease is frequently associated with parathyroid enlargement. Renal lesions have been found to accompany hyperplasia or tumors of the parathyroids in over 50% of the reported cases of osteitis fibrosa,[1] and parathyroid enlargement has been demonstrated in various forms of chronic renal disease[2, 3, 4] and in renal dwarfism.[5, 6]

The nature of this relationship is not well understood and it seemed of interest to approach the problem experimentally. In this brief report, it will be shown that significant increase in the volume of the parathyroids may be brought about in rats by experimentally reducing the amount of functional renal tissue.

Healthy white rats, weighing from 150 to 250 gm., and maintained on a mixed diet were used for the experiments. One kidney was removed through a lumbar incision, and a considerable portion of the opposite kidney was destroyed by thermo-cautery in 2 operations.

The chief symptoms noted were drowsiness, roughness of the hair and loss of appetite. Although 3 of the rats showed a transient gain of weight after the second cauterization, the weight later remained stationary or declined progressively.

At autopsy, the parathyroids with attached thyroid tissue were fixed in Zenker's fluid, and serially sectioned at 10μ. Their volume was determined by multiplying the combined areas of the section, as obtained with the planimeter from drawings projected at known magnification, by the thickness of the section. The volume was calculated for 100 gm. of rat. Since all of the operated rats lost weight before death, the calculations were based upon the "normal" weight at time of death as estimated from Donaldson's tables, and not upon the actual weight.

1 Albright, F., Baird, P. C., Cope, O., and Bloomberg, E., *Am. J. Med. Sci.*, 1934, **187**, 49.

2 McCallum, W. G., *Bull. Johns Hopkins Hosp.*, 1905, **16**, 87.

3 Bergstrand, H., *Acta med. Scandinav.*, 1920, **54**, 539.

4 Pappenheimer, A. M., and Wilens, S., *Am. J. Path.*, 1935, **11**, 73.

5 Smyth, F. S., and Goldman, L., *Am. J. Dis. Child.*, 1934, **48**, 596.

6 Langmead, F. S., and Orr, J. W., *Arch. Dis. Childhood*, 1933, **8**, 265.

The operated rats fall into 2 groups according to the time of survival. Those allowed to live for a period of 113 to 124 days following the second cauterization showed much more intense lesions of the remaining kidney tissue than did those in the group killed or dying before 46 days.

The data are summarized in Table I.

TABLE I.

Average Combined Volume of Parathyroids per 100 gm. of Rat.

	Volume cu. mm.	PE_m	Standard Deviation
Group A—Controls (9)	0.1441	0.0130	0.0551
Group B*—Early nephritics (5)	0.1679	0.0066	0.0197
Group C*—Late nephritics (5)	0.4117	0.0553	0.1659

*Calculations of B and C are based on estimated normal weights at time of death.

In spite of the small numbers of animals, the difference in the mean volume of the parathyroids between the control group and the later group showing the more severe renal damage, is statistically valid.† In the case of the earlier group, the difference is suggestive, but not conclusive, although in each rat, the parathyroid volume exceeded the mean volume of the controls.

One may reasonably conclude that the reduction of functional renal substance has led to a decided increase in the size of the parathyroids. The question arises as to whether this increased volume is due to enlargement of the cells or to their multiplication. Measurements of the nuclei in 2 diameters indicated that the nuclei in the glands of operated rats were larger than those of the controls. Thus, the mean diameters of 250 nuclei in the 5 "nephritic" glands were 8.0x6.2μ, as against 6.8x4.4μ in 200 "normal" nuclei. It could also be demonstrated that this alteration in nuclear size was attended by an increase in cytoplasmic volume. Mitotic figures were only occasionally found. It would seem that the increase in bulk in the "nephritic" parathyroids may be accounted for, in part at least, by the increased volume of both nucleus and cytoplasm.

† The following formulae were used in the calculations:

$$\sigma = \sqrt{\frac{\Sigma x^2}{n-1}}$$

$$PE_m = \tfrac{2}{3} \times \frac{\sigma}{\sqrt{n-1}}$$

$$PE_{m_1 - m_2} = \sqrt{(PE_{m_1})^2 + (PE_{m_2})^2}$$

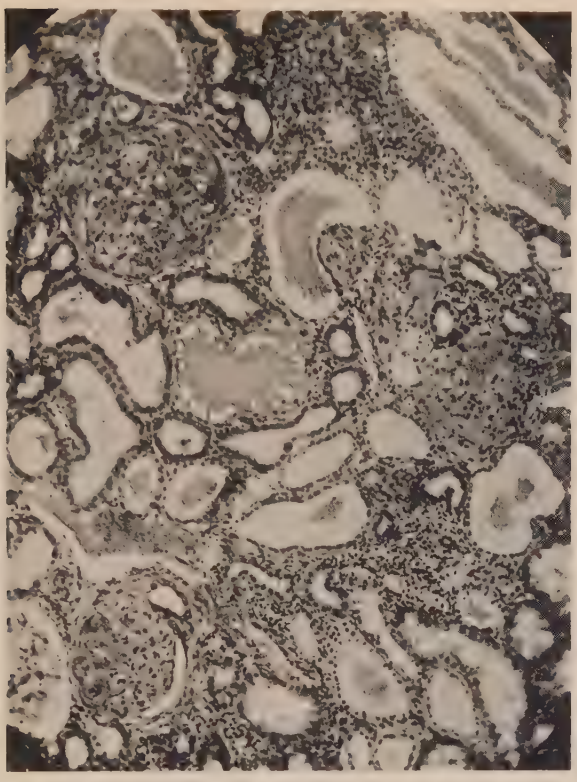


FIG. 1.

Rat AA2: Three glomeruli in section showing adhesions and partial hyalinization; dilatation of tubules with casts, interstitial fibrosis and lymphoid infiltration. × 100.

The pathologic alterations of the remaining kidney substance, in the later group, C, are diffuse and severe, simulating an advanced stage of glomerulo-nephritis (Fig. 1). At least 90% of the glomeruli are greatly enlarged, bloodless, the capillary loops distended with hyaline or granular material, the capsular space obliterated by adhesions. Often there is crescentic proliferation of epithelial cells. In many tufts, these changes have progressed to almost complete hyalinization.

The majority of the tubules are widely distended with dense hyaline coagulum. The epithelial cells are flattened, so that in some areas the tissue resembles thyroid. There is irregular interstitial fibrosis, with moderate lymphoid cell infiltration of the stroma. In a few of the larger arteries and in some of the arterioles, there is fibrinoid or hyaline material in the subendothelial tissue.

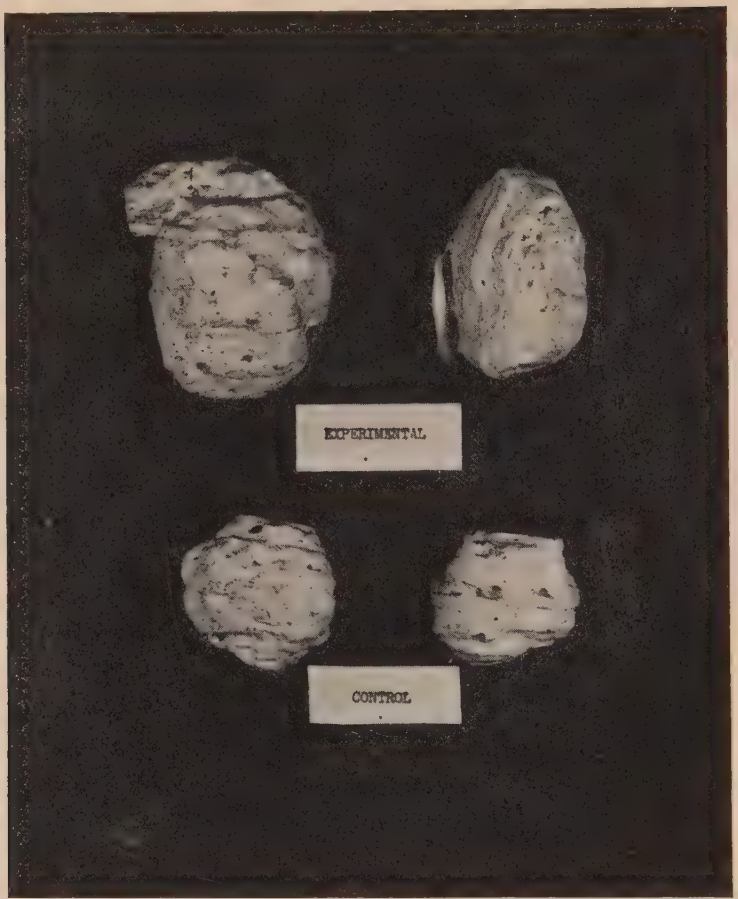


FIG. 2.

Wax reconstructions of parathyroids from Rat AA6 (experimental) and Rat 3B (control). Drawings at linear magnification of about 40.

With minor variations in intensity, this same picture is found in each of the 5 animals of the later group. Although the pathogenesis of the lesions is not clear, it is of interest that the cauterization leads to the gradual development of a severe diffuse nephritis, with glomerular lesions comparable to those of advanced human glomerulonephritis.

Such diffuse changes were not seen in the kidneys of group B, killed within 46 days of the last cauterization. The lesions in these rats were limited to the actual necrosis caused by the procedure, and did not involve to any extent the intact parenchyma. There was, however, a striking hypertrophy of both glomeruli and tubules.

as described in dogs by Allen, Bollman and Mason[7] after unilateral nephrectomy and partial resection of remaining kidney.

8035 P

Cardiovascular Studies in Patients with Single Functioning Lungs.*

A. M. MASTER, HARRY L. JAFFE, AND S. DACH.

From the Surgical Service of Dr. Harold Neuhof and the Cardiovascular Service, Mount Sinai Hospital, New York City.

On the service of Dr. Harold Neuhof at The Mount Sinai Hospital, New York, we have had the unique opportunity of making cardiovascular studies of patients with one functioning lung. Multiple thoracoplasties were performed by Dr. Neuhof for unilateral chronic empyema, pulmonary tuberculosis or lung abscess. The lung on the non-affected side was normal. Table I.

Dyspnoea on exertion, not at rest, was universally present. There were no orthopnea and cyanosis. The hearts were normal, although perhaps displaced and slightly rotated. This was judged by physical examination, teleoroentgenogram, fluoroscopy and electrocardiogram. In 2 cases there was a tendency to right ventricular preponderance on the electrocardiogram, probably the result of the long standing previous pulmonary disease with slight rotation of the heart. In one case there was a definite left ventricular preponderance.

The pulse rate was always rapid. The blood pressure was normal. The respirations were usually 20 per minute, occasionally 28 per minute, but the slightest exertion increased the respirations. The vital capacity was markedly diminished, ranging between 1200-2700, the normal being 3500-4500. The venous pressure by the direct method[1] was definitely elevated on the involved side in one case. The velocity of the blood, measured from the arm to the tongue[2] or the arm to the lung,[3] was definitely increased, that is, the

7 Allen, R. B., Bollman, J. L., and Mann, F. C., *Arch. Path.*, 1935, **19**, 174.

* This investigation was made possible by grants from Mr. Herbert H. Lehman, Mrs. Charles and Mr. Frank Altschul.

1 Taylor, F. A., Thomas, A. B., and Schleiter, H. G., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 867.

2 Fishberg, A. M., Hitzig, W. M., and King, F. H., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 651.

3 Hitzig, W. M., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 935; Miller, H. R., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 942.

TABLE I.

Single Functioning Lung.

	W. R. 16 yrs. Multiple Thoracoplasty Chr. Empyema	D. B. 25 yrs. Multiple Thoraco. Tbc.	*B. K. 27 yrs. Multiple Thoraco. Tbc.	W. K. 22 yrs. Lobectomy Chr. lung abs.	J.G. 45 yrs. Multiple Thoracoplasty Chr. empyema cavity	
Affected side	Right	Right	Left	Left	Left	
Kyphoscoliosis	Marked rt.	Marked rt.	None	None	None	
Mediastinum	Fixed	Fixed	Fixed (to left)	Fixed	Shifting	Fixed
Dyspnoea on exertion	Present	Present	Present	None	Marked	None
Orthopnea	None	None	None	,,	Present	,,
Cyanosis	,,	,,	,,	,,	None	,,
Heart	Normal	Normal	Normal	Normal	Normal	
Heart rate	80-90/min.	80-96/min.	100-130/min.	92-100/min.	90-110/min.	90/min.
Respiratory rate	20-26/min.	20/min.	20-28/min.	20-18/min.	20/min.	20/min.
Bl. pressure	120/80	110/78	110/60	140-150 / 90-100	120/80	
Vital capacity	1200	1700	1200	2700-1600-2100	1000	1500
Venous pressure	R.6-10	R.-7½	L.-8	L. 12-27; R. 3½ 7	L. 9; R. 9	R. 1
Circulation time						
Arm to tongue	12	10¾	7-9	10-11	9¾	
Arm to lung	5	5-7	2½-3½	4	5	
Electrocardiogram	L.V.P.	R.V.P.	T_2 low	Normal	R.V.P.	
Exercise tolerance	Reduced	23/27	T_3 inverted	15/24		10/25
Hemoglobin	65%	90%	80%	72%	55-60%	66%
O_2 content				17.7		17.7
O_2 capacity				19.0		18.8
% saturation				93.3		94.2
Bl. volume per Kg.		86 cc.				73 cc.
Basal metabolism		—14, —11, —2				+7, +2, +5

*Female.

circulation time was decreased. In every case the mediastinum was fixed except that a shifting mediastinum was present for a short time in one patient (J. G.). On inspiration the entire mediastinum moved to the left and on expiration to the right. This produced decided embarrassment to the patient, *i. e.*, marked dyspnoea and orthopnea, even while he was in bed.

The basal metabolic rates were normal in the 2 patients tested.

The oxygen content and capacity of the arterial blood was normal in the 2 patients in whom it was studied.

The exercise tolerance as quantitatively measured by the two-step test[4] was definitely diminished.

It appears that a man with only one functioning lung is comfortable while at rest; the blood velocity is speeded up and the heart rate increased, resulting in a greater flow of blood through the remaining lung and therefore resulting in adequate oxygenation for metabolism at rest, despite the very low vital capacity. However, the additional burden involved in even moderate exertion suffices to produce symptoms of cardiorespiratory insufficiency.

[4] Master, A. M., and Oppenheimer, E. T., *Am. J. Med. Sci.*, 1929, **177**, 223.